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WINTER INJURY OF ALFALFA[1]

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INTRODUCTION

In the spring of 1925 L. E. Melchers showed the writer a serious injury of the crowns and taproots of 1-year-old alfalfa plants grown in Kansas. The injury, which he later described,[3] was characterized chiefly by browning and decay of the outer part of the taproot beneath the crown. (Pl. 1, A and B.) The circumstances accompanying the occurrence of the injury suggested as its probable cause some adverse climatic condition during the winter months. It was found occasionally in several other States in the spring and summer of 1925. In the spring of 1926 similar injury was found by the writer to be widespread and often severe in southern Wisconsin. Here it occurred in plants of all ages and was always present in some of the surviving plants in fields where more or less of the stand had died from winter-killing. In the spring of 1926 J. L. Weimer found in Kansas much root injury, which, upon examination, appeared to be exactly like that in Wisconsin. A detailed description of the outward appearance of these injured plants has been published by Weimer (*7*),[4] who suggested that the origin of the lesions might be winter injury. This term appears well chosen and will be used here inclusively to designate all injuries that appear to be due at least in part to cold during the winter season.

During 1926 Weimer and the writer collected as widely as possible alfalfa plants showing evidences of winter injury in all degrees of intensity, and the writer began an examination of this material to determine as far as possible by histological methods which tissues first show injury and how the later conspicuous lesions develop. Before this work was completed winter injury appeared again in the spring of 1927, not only in southern Wisconsin, where it was discovered and material collected earlier than in 1926, but also in Kansas, Iowa, and other States, where further collections were made and examined. Thus the following description is based on material collected in both 1926 and 1927 in the central Mississippi Valley.

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[2] The writer is indebted to many persons for aid in the execution of work presented in part in this paper—especially to Dr. J. L. Weimer, of the Office of Vegetable and Forage Diseases, for many collections of material; to Prof. E. J. Kraus, of the University of Wisconsin, for suggestions in histological studies; to Dr. G. H. Conant for aid in the study of seedling anatomy; and to Dr. Valdimer Skoric for aid in the preparation of the drawings.

[3] MELCHERS, L. E. CROWN AND ROOT ROT OF ALFALFA (UNDET.). U. S. Dept. Agr., Bur. Plant Indus. Plant Disease Rptr. 9:54. 1925. [Mimeographed.]

[4] Reference is made by number (italic) to "Literature cited," p. 211.

The work has been done not only for the purpose of facilitating the early recognition of the injury in experimental demonstrations of its cause, but also for the purpose of determining its relation to bacterial wilt, which often destroys winter-injured plants.

Before describing in detail winter injury in the crown and taproot of the alfalfa plant, it is necessary to trace the development of the tissues in which the injury occurs.

DEVELOPMENT OF THE ALFALFA PLANT

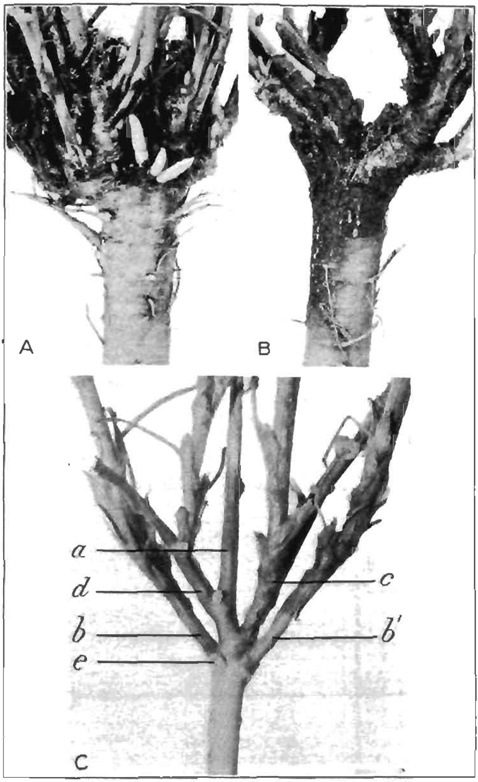
GROWTH CHARACTERISTICS

The general appearance of the alfalfa seedling has been so well described by Compton (*1*) and others that no new description is needed here except in so far as this seedling forms a part of or gives rise to the perennial part of the plant with which we are especially concerned. The seedling root is surmounted by a hypocotyl about 1 cm. long, distinguished from the root by the absence of root hairs. After secondary growth begins and the root hairs on the taproot are lost it is no longer possible to distinguish the hypocotyl readily, and in this paper it will be considered as a part of the upper end of the taproot. The upper end of the hypocotyl is marked by the bases of the cotyledonary leaves, which, together with the first unifoliolate leaf, are the first foliage of the seedling. With the development of the single seedling stem, the first trifoliolate leaf arises above and nearly opposite to the unifoliolate leaf, and other leaves arise in characteristic order.

The branching from the primary axis of the plant, leading to the formation of the characteristic crown, usually proceeds in the following manner: The first stems from the primary axis are usually three in number developing almost simultaneously from the axils of the cotyledonary leaves (pl. 1, C, *b* and *b'*) and of the unifoliolate leaf (pl. 1, C, *c*). In addition to these a stem may develop from the axil of the lowest trifoliolate leaf (pl. 1, C, *d*). The bases of these three or four stems usually constitute the largest branches of the crowns of old plants.

In addition to these stems from axils of leaves, in vigorous plants other stems may arise from buds that are not axillary in origin and form a part of the crown or perennial portion of the stem structure of the plant. The first nonaxillary bud usually arises between the bases of the stems that have arisen from axils of the cotyledons and in a position opposite to that of the first unifoliolate leaf. (Pl. 1, C, *e*.) In fact, if the plant is very vigorous several buds may arise almost simultaneously in this region, and later from any point in the circumference of the plant at or a little above this level. The numerous small stems of which large crowns are so largely composed arise either in an apparently adventitious manner in this region or from the axils of scales and leaves on the bases of stems. The number and habit of growth of these stems vary greatly in different varieties of alfalfa, and within a variety the number is largely dependent on the vigor of individual plants.

As might be expected, young alfalfa plants in thickly seeded fields rarely show the symmetrical development described in the preceding paragraphs. In less vigorous plants some of the first buds are often suppressed and die, leaving the crown one-sided and irregular. If a



A.—Crown and portion of taproot of an uninjured alfalfa plant from a 4-year-old field at Madison, Wis. (October, 1926).

B.—Crown and portion of taproot of a plant similar to that shown in A except that it suffered severe injury in the preceding winter. The blackened condition of the crown and upper part of the taproot and the absence of buds from the base of the crown are evidences of this injury.

C.—Symmetrical crown of a hardy alfalfa plant at the end of its first winter: *a*, The first seedling stem; *b* and *b'*, stems from the axils of cotyledons; *c*, stem from the axil of the unifoliolate leaf; *d*, stem from the axil of the first trifoliolate leaf; *e*, first bud from the base of the crown arising between the stems from the axils of the cotyledons and opposite the stem from the lowest seedling bud.

seedling becomes too deeply buried by soil or even by a mass of foliage close to the ground, the first crown branches may develop from buds higher up on the seedling stem. The position of the crown seems to be determined largely with reference to the soil level. If at any time a plant is buried, buds near the new ground level will form a new crown above the first one; but if soil is washed away from around the plant, buds do not arise from the exposed root to form a new crown at a lower level.

It should be noted that the term "crown" is used here as it has been used by Stewart (*6*) to designate the perennial part of the alfalfa plant derived from stems. It will be shown later that the term thus restricted applies to a region with distinctive histological and morphological characteristics.

ANATOMICAL STRUCTURE OF THE TAPROOT

As a further background for the description of the injuries found in the taproot and the crown of the alfalfa plant, the development of the tissues involved will be briefly traced. The anatomy of the primary structures of the seedling has been studied by Compton (*1*), who was chiefly interested in tracing the transition from the triarch root to the stem structure in the upper part of the hypocotyl. With the details of this transition the present study is not concerned, but it is important to note that in the hypocotyl the xylem groups are separated by parenchyma, which at the upper end sometimes forms a pithlike center of the structure. This central parenchyma is separated from the true pith of the stem by a node at the base of the first unifoliolate leaf where the vascular elements are closely packed at the center of the stem, and the interfascicular cells near the center are elongated axially with pointed ends. The pointed cells usually become lignified, separating the pith of the stem from parenchyma in the center of the root with a woody plate. The extent of lignified tissue at the center of the axis varies greatly. In a Peruvian alfalfa seedling with its crown above the soil the lignification may extend for some distance down into the hypocotyl, but usually it appears to be confined to the node.

With the addition of secondary xylem to the vascular cylinder, the cells of the central parenchyma of the hypocotyl and possibly of the upper part of the root, which can hardly be distinguished from the hypocotyl, begin to enlarge. At first they are round with intercellular spaces, but later they become irregular in shape and more crowded. Parenchyma of the early secondary growth expands like that of the primary structure. Cells contiguous to vessels may divide with walls tangential to the proximate vessel or group of vessels. In vigorous plants more or less expansion of the central parenchyma seems to continue during the first summer of the plant's growth.

In consequence of this expansion of the central parenchyma, the primary vascular strands and the earlier vessels with accompanying fibers of the secondary growth are pushed into more or less sinuous courses, weaving back and forth and anastomosing in the pithlike center of the root. This change in position of the vascular tissue suggests comparison with an apparently similar distortion described by Gravis (*2*) in the vascular tissue of *Crinum capense*. In Crinum the leafy crown of the plant is drawn down into the soil by a con-

spicuous shortening of the hypocotyl. Gravis found the shortening to be due to the radial expansion and axial shortening of the parenchymatous cells composing the cortex. Now, in fact Rimbach (*4*) has described a marked shortening of that part of the alfalfa root derived from the hypocotyl, which seems from his account to have taken place during the time when this expansion of the central parenchyma was in progress. A comparison of the figures given by Gravis with longitudinal sections of alfalfa roots suggests that if these roots have shortened as described by Rimbach, the mechanism that brought it about may well have been the radial expansion of the central parenchymatous cells, similar to though less in amount than the radial expansion of cortical cells in Crinum. However this may be, the fact that concerns us here is that the upper part of the taproot, especially that derived from the hypocotyl, is distinguished from the rest of he taproot by having at the center abundant parenchyma interspersed between groups of vessels and fibers, which are irregular in course and anastomose freely.

A feature of the primary structure of the hypocotyl that has not been mentioned in the literature is the presence of four bundles of fibers opposite the phloem groups. These fibers are better developed at the upper end of the hypocotyl and usually do not begin to lignify until after secondary growth is laid down, though they are distinguishable by size and position very early. These may be the characteristic pericyclic fibers found in many species of the Leguminosae (*5*).

ANNUAL RINGS OF GROWTH IN THE TAPROOT

In the upper part of the taproot that part of both the wood and the phloem produced by the cambium in the autumn of each year is so different in character from that produced in the summer that annual rings of growth can be discerned similar in character to those in the wood of trees. The autumn growth of wood is distinguished by having vessels of smaller diameter and by the presence of small regular parenchymatous cells with few or no interspersed fibers between the vessels. (Figs. 1 and 2.) These growth rings might be observed by the unaided eye as readily as those in wood if it were not for the fact that the fibers in each year's growth are usually in distinct groups separated by vessels that break the wood into several apparent layers each of which may easily be mistaken for an annual ring.

The annual ring of growth in the phloem is usually as well defined as that in the xylem, though its annual increase in thickness is comparatively small. In the spring and early summer fibers more or less grouped or massed together are laid down opposite each vascular bundle as though to form a new bundle cap. Later a group of sieve tubes and phloem parenchyma with few or no interspersed fibers is laid down beneath the bundle of fibers. When a bundle of fibers is produced in the spring of the following year, the sieve tubes of the preceding year gelatinize and collapse under a pressure that sometimes also crushes interspersed parenchyma. Thus the annual increment in the phloem consists of a bundle of fibers and a group of sieve tubes and parenchyma, which may be somewhat crushed if new fibers have been deposited between it and the cambium. Thus annual rings of growth can often be seen and counted in the phloem of the root as readily as in the wood. Because of these annual rings

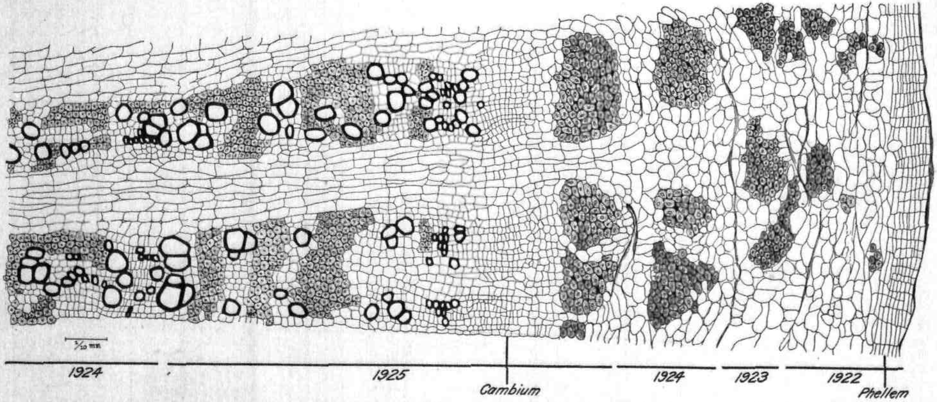


FIG. 1.—Outer portion of a vascular bundle from the upper part of a 4-year-old alfalfa taproot developed from the hypocotyl of the seedling. In this bundle each annual addition in growth is easily distinguished both in the xylem and in the phloem. In the xylem the spring wood has relatively large vessels and abundant fibers. The late autumn wood has few or no fibers and has small vessels surrounded by xylem parenchyma. The end of each annual cycle is marked by the abrupt transition from small to large vessels. In the phloem a bundle of fibers is laid down in the spring, when the xylem is also producing abundant fibers. Sieve tubes and parenchyma are produced in late summer and fall, only to be crushed as soon as the next fibers are produced in the spring. The inner portion of this bundle is shown in Figure 2

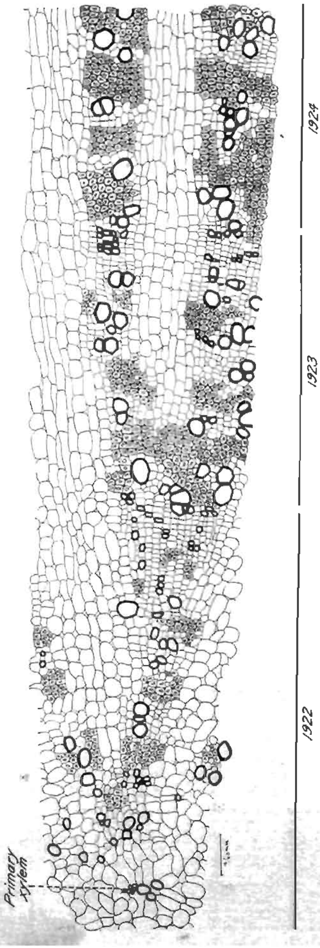


FIG. 2.—Inner portion of the vascular bundle shown in Figure 1. (See legend for Figure 1.)

the year and season in which any portion of the root was laid down by the cambium can usually be determined.

It may be added here that the wood and the phloem of the first year, and sometimes the phloem of the second year, have peculiarities that when recognized facilitate the determination of the age of roots and that are also important in the discussion of the pathological morphology that follows. The wood laid down by the cambium in the first year contains relatively fewer and more scattered fibers than the later growth. The same condition obtains in the phloem where very early in its activity the cambium often lays down a few scattered fibers separated by parenchyma from a later and usually larger group of fibers. When several years of added growth have compressed the early tissue, the fibers of the first year may appear aggregated into two distinct groups. These two groups are usually not completely separated by crushed tissue, or are separated by a zone so narrow and irregular in comparison with the zone of thin-walled tissue in the phloem of subsequent years that no difficulty in determining age need be occasioned by this peculiarity. The same tendency toward the production of two groups of fibers in the phloem of the second year has also been observed, though rarely. It has not been seen in the phloem of later years.

It should be noted here that alfalfa roots from certain regions offer great or even insuperable difficulties in age determination. In roots over 10 years of age from the dry land of western Kansas the annual increment in diameter is often small and uniform in character. Collections from southern California, where the plants do not become dormant in the winter, also fail to show easily distinguishable bands of autumn growth. With these exceptions, however, among 40 collections of known age in the Mississippi Valley from Alabama to Minnesota only 1 collection failed to show internal evidence of age corresponding to the known age of the plants. In this 1 exceptional collection from Manhattan, Kans., two distinct annual rings were deposited in the second year.

ANATOMICAL STRUCTURE OF THE CROWN

The alfalfa crown is here described as that part of the stem structure that is perennial. The ability of the crown to withstand the winter may be due in part to its protected position, but it also seems to be associated with certain peculiarities in secondary growth that should be noted. Even in its primary structure the stem base suggests in some characteristics a transition between root and stem. At the base there is usually a well-defined endodermis (fig. 3), which is less easily distinguished and finally disappears at higher levels. The fascicular and interfascicular cells become heavily lignified, forming a cylinder of strong supporting tissue.

The cambium is very active in the stem bases, especially in the first axillary stems, which will form the large crown branches. When the cambium begins to produce secondary growth the endodermis becomes a phellogen. The portions of the endodermal cell walls inside the thickenings, if any have been laid down, expand conspicuously, and almost simultaneously two or three cross walls are laid down interior to these thickenings. Hereupon the epidermis dies, separates from the cortex, and forms the brown membranous covering often seen on young stem bases early in spring. The phellem

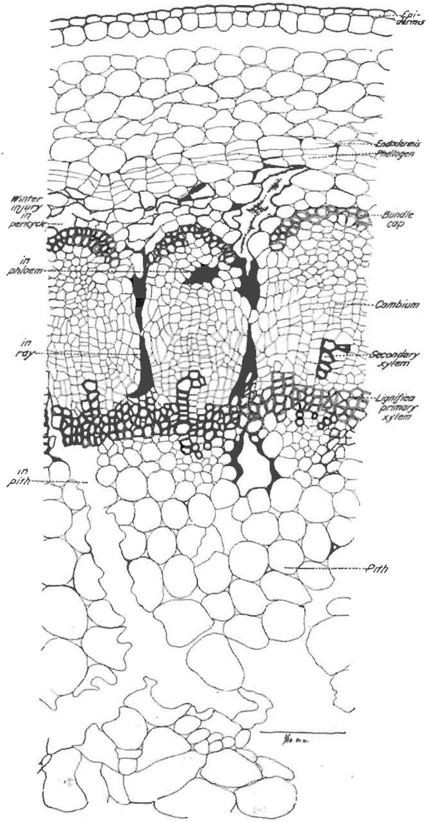


FIG. 3.—Portion of the cross section of a young alfalfa crown stem or stolon showing winter injury. Collected at Madison, Wis., Apr. 6, 1927. Injury occurs: In pericycle, shown by rifts between cells and by discolored cell walls; in the phloem, a black mass in the central bundle; in the vascular rays, extending as black masses of dead cells separating bundles, except at the cambium; in the pith, as broken places surrounded by blackened remains of cells near the primary xylem, and by rifts in the tissue extending radially from the center

originating from the endodermis becomes the effective outer covering, both in the large crown branches from axillary buds and in the small stems. The cambium, which in the beginning may have made some addition to the heavily lignified central cylinder of the stem, begins sooner or later to lay down tissue that is indistinguishable from that which is being produced in the root. The lignified interfascicular cells give way to wood rays, which may store starch, and the structure appears to function in all ways like a root except that it seems able to give rise to buds. The manner in which these buds arise has not been studied. The transition from stem to root structure may occur suddenly, or gradually, or irregularly about the stem. After the change from stem to root structure has been made, the cambium rarely reverses the transition and produces stem structure again, though occasionally small islands of lignified tissue occur in the root structure. Thus occasionally large crown branches buried in the soil for several years become so rootlike in character that their origin is revealed only by the presence of pith and the lignified stem structure at the center.

METHOD OF STUDY

COLLECTION OF MATERIAL

A large part of the plants examined in the beginning of this study was collected in the course of an investigation of bacterial wilt with which some of them were infected. When in the spring of 1926 lesions that are here interpreted as winter injury began to become visible in wilt-infected plants, it was not always possible to distinguish with certainty some of the discolorations caused by winter injury from those caused by the parasitic bacteria. It was soon evident that a thorough knowledge of the character of winter-injury lesions must be gained before the two pathological conditions could be always distinguished. In the last days of April, 1926, the collection of plants showing what was regarded as winter injury was begun. At this time plants were beginning to grow and lesions were not only discolored and easily visible but the injured tissue when sectioned stained in a characteristic manner by the method described below. From this time onward the development of the injuries and the growth response of injured plants were followed through the growing season.

In 1927 it was possible to follow the development of lesions from the time frost left the ground through the spring. Fortunately for this work, heavy ice sheets formed in late winter in some alfalfa fields in southern Wisconsin, killing a large part of the stand. When the ice began to melt, plants that had been buried in ice so long that their death or injury was anticipated were chopped from the frozen ground and examined. In this way winterkilling and winter injury were traced from the time the plants thawed until early summer.

In the following pages the characteristic location of injury of different degrees of intensity and in plants of different ages will be traced. Finally, the evidences of injury as they may be seen with the aid of razor sections will be traced from the beginning to the appearance of characteristic discolored lesions.

PREPARATION OF MICROSCOPIC SECTIONS

From the very beginning of this work it was necessary to make and stain microscopic sections from many plants. A method suggested by E. J. Kraus gave the most satisfactory results. The segments of roots were embedded in parowax in the usual manner. If the material could not be sectioned at once without tearing the sections, the cut ends of the embedded roots were placed in water until they were sufficiently softened to cut easily. A soaking varying from 6 to 24 hours was usually enough. A well-sharpened microtome knife of excellent quality was used, but even with this it was not often possible to cut more than a dozen sections at one place on the blade before the edge was dull and the sections began to tear. With proper care, however, it was possible to get such perfect sections of almost any root that any mechanical injuries produced in roots by freezing were not confused with disarrangement of tissue in the preparation of the sections.

It is fortunate indeed that the method used in staining the parasitic bacteria served to differentiate injured tissue. The method of differential staining used was as follows: The segments of roots were usually fixed in formal-acetic alcohol. The sections were first stained in dilute safranine long enough to give a red color to vessel walls but not to walls of parenchymatous cells. Overstaining in safranine prevented the desired action of the stains that followed it. After standing in water a few minutes, the sections were stained according to Gram's method as described in manuals of bacteriology, except that each slide was dipped in water after immersion in the iodine solution to prevent the appearance of iodine crystals after dehydration in alcohol. It should be noted that the gentian violet and the iodine solutions as usually prepared for this method are at least three times as concentrated as necessary, and that dilutions can be used with equal success, and even to great advantage if the sections contain much starch, which will be blackened by the strong iodine. Diluted stains do not keep and are useless after a few days. Instead of diluting the aniline gentian violet and iodine it is often possible to reduce the time in them to 10 seconds with satisfactory results. Following immersion in the iodine solution, the slides were dipped in absolute alcohol until the stain no longer appeared to run from the sections when the slide was lifted. They were then flooded with a saturated solution of Orange G in clove oil to which about 20 per cent of absolute alcohol had been added. This solution stained the walls of the parenchyma a deep yellow and also removed surplus gentian violet left by the alcohol. If the gentian violet was not removed sufficiently by the clove oil in one or two minutes, the slide was returned to the absolute alcohol for a few seconds for further clearing, and again flooded by clove oil. It was then cleared in xylol and mounted in the usual manner. In sections treated in this way the parasitic bacteria (*Aplanobacter* [*Phytomonas*] *insidiosum* L. McC.) stained a deep blue, while the walls of parenchyma that had been winter injured appeared black, slate gray, or even red, depending on the time that had elapsed since freezing, as will be explained later.

This method appears to be adequate for differentiating the pathological conditions resulting from winter injury from those resulting from bacterial wilt, even when both occur together in the same part

of the plant. It has been used exclusively in the preparation of sections from which drawings have been made.

MICROSCOPIC APPEARANCE OF INJURED TISSUE IN ROOTS AND CROWNS

In microscopic sections of injured plants the presence of injury is disclosed by the disarrangement of cells and by the way in which they stain. The position of the injury is definitely characteristic, as will be shown later. Two types of cells, parenchyma and vessels, are chiefly involved.

INJURY IN PARENCHYMATOUS TISSUE

From an examination of Figures 1 and 2 it is readily seen that the pithlike cells at the center of the root and the wood and phloem rays together with abundant phloem parenchyma form a continuous mass of similar cells broken only by the cambium, which is not always visibly different from the tissue that it has produced. In this cell mass is found the larger part of the injury that gives rise to the visible lesions on the plant.

In plants injured during their first winter the cells that show injury first are usually located in the phloem and the phloem rays. The injury may occur wholly in the large cells exterior to the bundle or groups of fibers. (Fig. 4.) Oftentimes the dead cells form a sheath surrounding the root, whereupon all cells exterior to it die and slough off, after which the root has a roughened surface. Injury in the phloem is usually accompanied by more or less necrosis of the large cells at the center of the root and also by lesser injury in the wood rays. (Fig. 5.) Plants injured during their first winter may survive even when little root tissue besides the cambium and the outer portion of the vascular bundles (fig. 6) is left alive below the crown.

In plants injured during their second or any subsequent year the injury usually appears first in the parenchymatous cells of the phloem and phloem rays, as in 1-year-old plants, but the characteristic position of the dead cells is somewhat different, being inside rather than outside the last group of fibers in the phloem (fig. 7); and while the necrotic ring may cross the phloem rays, as described previously, it usually follows inward through the cambium along the wood rays to a depth not exceeding the last annual increment in growth. Thus the cambium is broken at frequent intervals, but the injury does not extend all the way to the center of the root so frequently as in younger plants. The pithlike center is injured as in younger plants, though perhaps not quite so abundantly with increasing age. Accompanying the injury already described and sometimes independent of it, there may be a narrow more or less continuous zone of necrotic cells beneath the phellogen close to the characteristic layer of cells containing crystals. Since the inner walls of the cells containing crystals stain much like those of injured cells, it is sometimes difficult to distinguish true injury in this region. (Fig. 8.)

In the crown stems injury is altogether similar to that in the roots. (Fig. 3.) The large cells beneath the phellogen derived from the endodermis and the large central pith show injury first. The ray cells are easily destroyed and the bundles become separated by dead

cells as in the root, though they may be held firmly together mechanically by the inner band of primary xylem. Phloem parenchyma may be injured as in the root. When the crown has begun to be rootlike in character it suffers injury like that of roots.

The precise nature of the action of frost upon the alfalfa root, which gives rise to the injuries described here, can not be determined

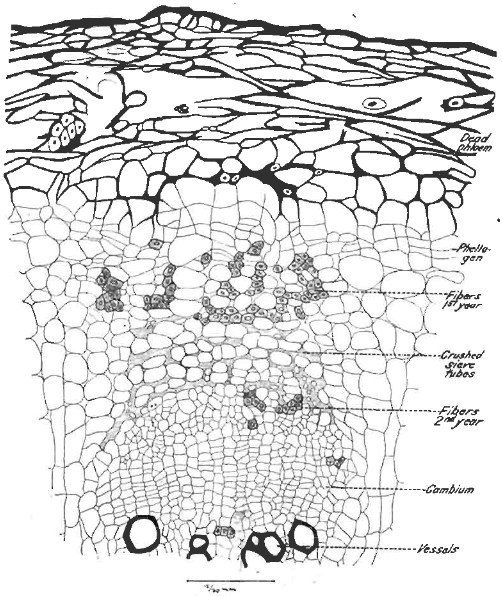


FIG. 4.—Cross section of the outer portion of one vascular bundle of an alfalfa root showing characteristic injury inflicted during the first winter. Grimm alfalfa planted in Doniphan County, Kans., in 1925, and collected Apr. 17, 1926, after it had begun vigorous spring growth that had crushed the phloem produced in 1925. The injury occurred in the phloem exterior to the group of fibers, not only in the bundle, as shown here, but also in the phloem rays, resulting in a ring of dead cells all around the outside of the root. Cells exterior to the injured ring died and collapsed. Meristematic activity of cells below the injured ring was forming a new phellogen layer that would protect the root when the dead tissue decayed

merely from observation of the injury. It appears, however, that the injury is always found where cells have been separated by rifts along the middle lamellae. These rifts when extensive may result in the physiological isolation and death of tissue. Usually, however, it appears that the rifts are only incidental to injury of a very different

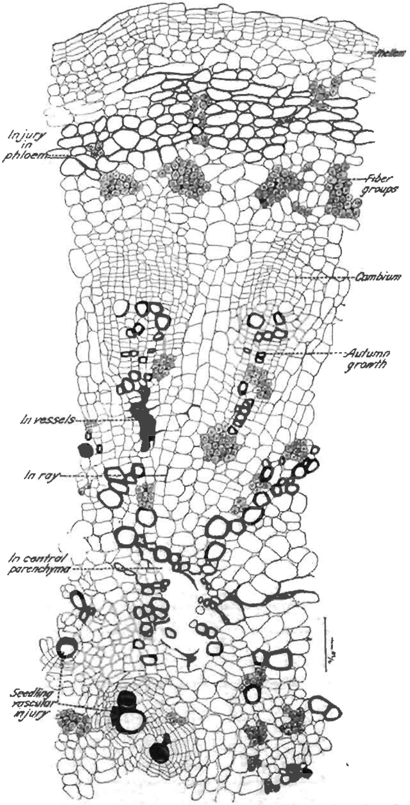


FIG. 5.—Sector of the cross section of a small alfalfa root of a nonhardy variety showing injury incurred during its first winter. Planted Aug., 1925, at Madison, Wis.; collected May 14, 1926, after growth had begun at the cambium. Injury occurred in the phloem like that shown in Figure 4, but no meristematic growth of the cells beneath the injury had begun in this case. Winter injury is shown also in the vascular bundles, in the wood rays, and in the central parenchymatous tissue. The vessels at the center of the root around which abundant cell division had taken place were injured during the previous summer. Such seedling injury is very common

character from the wounding of the tissue that these rifts occasion. This injury expresses itself by a deposition of material in and outside of cell walls. This material gives to these cells the staining characteristics previously described. The cells thus injured do not divide in response to the injury, but when located near the exterior

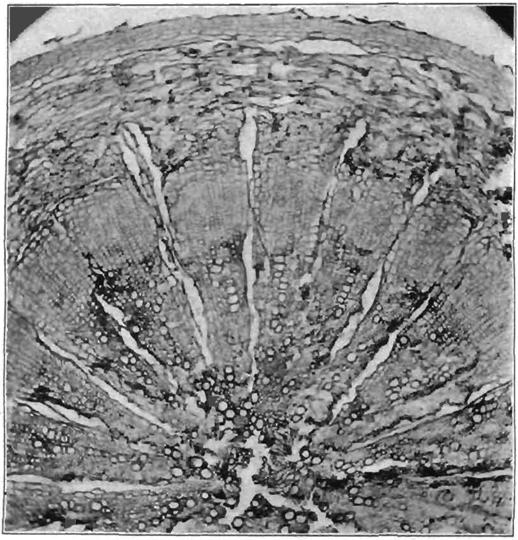


FIG. 6.—Photomicrograph of a portion of a section of the upper part of the taproot of an alfalfa seedling that had suffered severe winter injury during its first winter. Collected at Madison, Wis., Mar. 30, 1927. All tissue except fascicular cambium and adjacent immature cells appear to have been killed. The groups of living cells in the bundles had begun to expand and mature to furnish vascular connection to the buds above. The dark-staining material characteristic of winter injury occurs more or less abundantly at the margins of open spaces bordered by living or injured cells

of the plant are usually cut off eventually from the uninjured tissue by the deposition of suberin in the walls of cells located between them and the cambium. From this behavior the writer infers that the injury is primarily a direct effect of freezing, and not primarily or necessarily a consequence of the mechanical separation of cells, which appears also to be caused by the cold.

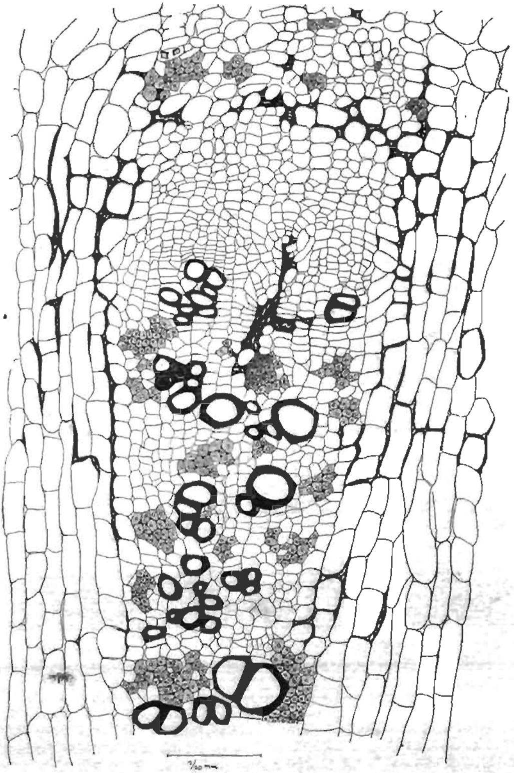


FIG. 7.—Cross section of the outer portion of a vascular bundle of the root of an alfalfa plant injured during its third winter. Grimm alfalfa collected at Monroe, Wis., May 6, 1926. The drawing includes nearly all the addition to the bundle in its third year. The injury represented chiefly by heavy lines indicating cell walls in which dark-staining material is deposited is situated largely in the parenchymatous cells of the phloem inside the innermost group of fibers and in the phloem and the wood rays. Injury in the vascular bundle is shown by a group of disorganized parenchymatous cells in the autumn growth and by two plugged vessels near the disorganized cells. This drawing should be compared with Figure 4, which represents a similar characteristic injury in a 1-year-old plant, and with Figure 9, which represents a less usual type of injury in a 3-year-old plant

INJURY IN THE VASCULAR BUNDLES

Injury in the vascular bundles is limited almost exclusively to cells produced during the previous growing season. It is first discerned in early-spring collections by the presence, in the lumina of vessels, of granules that stain like the walls of necrotic cells. Frequently one or more contiguous parenchymatous cells stain like injured cells in the phloem. If fibers are close to injured parenchyma, they are usually shrunken and stain deeply with safranine. Later in the summer injured vessels become plugged with a gumlike material (fig. 9), unless perchance they are in the center of a necrotic area. Tyloses sometimes appear, but they are always very small, never reaching a diameter equal to one-half that of the lumen of the vessel. Tyloses have been observed only in vessels of injured tissue. The distribution of plugged vessels in the outermost ring of growth shows

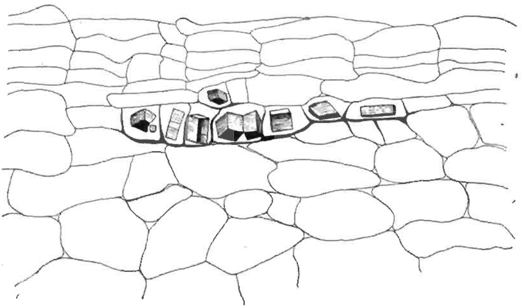


FIG. 8.—Crystals in cells situated beneath the phellem. The inner walls of these crystal-containing cells are much thickened and often stain like the walls of injured cells. Thus they are easily mistaken for injured cells, especially since the crystals are not often conspicuous

great variation in different plants. Sometimes plugging is found chiefly in the largest vessels, sometimes chiefly in vessels of autumn growth, and at others in scattered vessels of different size and age. In nearly all the collections from Wisconsin, but not in those from Kansas, whenever there is injury in the last annual ring, a few small vessels of autumn growth in each ring previous to the last have a staining reaction like injured parenchymatous cells and may also be shrunken and separated from adjoining cells.

In 1-year-old plants injury in vessels is associated almost always in varying amount with injury in parenchyma. In older plants it is on the whole less abundant and severe.

The origin and the composition of the material with which vessels become plugged have not been examined. In appearance the material resembles that formed in vessels of plants infected with bacterial wilt. Similar material has been found in vessels of plants into which

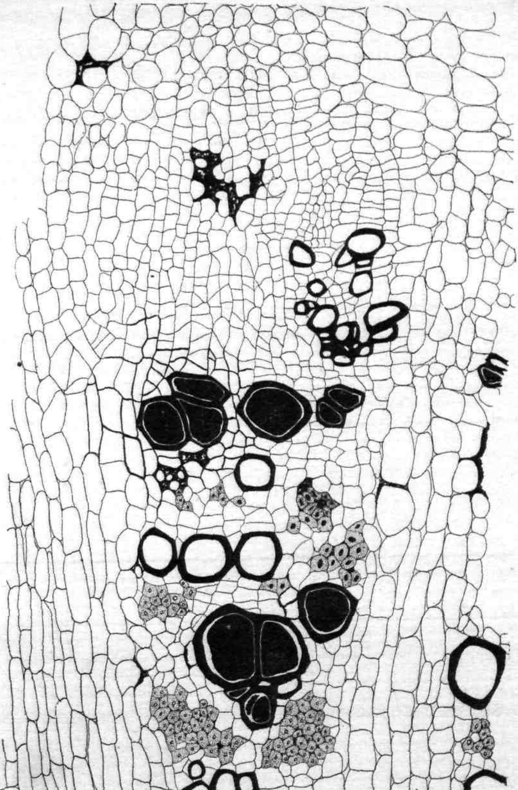


FIG. 9.—Cross section of the outer part of a vascular bundle of the root of an alfalfa plant injured during its third winter. Grimm alfalfa collected at Monroe, Wis., May 6, 1926. The drawing includes all of the addition to the bundle during its third year. This root and that shown in Figure 7 were collected from the same field. The injury represented here is less common than that illustrated in Figure 7. It occurs almost wholly in the bundle, and is shown by the plugging of vessels and the deep-staining walls of some of the adjoining parenchymatous cells represented by heavy lines. A small group of dead cells occurs near the center of the cambium of the bundle as in Figure 7, with which this drawing should be compared

dilute acids have been introduced through cut stems. Presumably the material is the product of living cells contiguous to vessels, and since in some cases it is evident that some of these have been injured, it is not unlikely that some product from them is the inciting cause of the deposition.

DEVELOPMENT OF WINTER INJURY IN RELATION TO WINTER-KILLING

Now that the character and position of winter injury in surviving plants have been described, it is of interest to trace the development of evidence of winter injury in several Wisconsin fields in the spring of 1927. On February 24 one collection was made from a 4-year-old field of Grimm alfalfa on which large ice sheets had remained for weeks. At that time the last of the largest ice areas was melting, and inasmuch as no severe freeze followed during the spring, it is assumed that all or nearly all of the damage found later had been done previous to that date. A number of plants were cut from frozen ground beneath the ice where winterkilling or severe injury was anticipated. When the plants were thawed the upper parts of the taproots looked more or less water-soaked, but none of them was conspicuously softened. These roots were embedded and sectioned. Sections of some of the plants showed tissue almost completely disorganized; rifts extended the entire length of the rays, the larger groups of parenchymatous cells had separated into loose aggregations, and the cells of the cambium region were collapsed, though coherent. In some plants nearly all of the tissue except the cambium was disorganized, while in others rifts were found only along the rays. No unusual staining reaction of injured cells was found.

On March 9, when the frost was out of the ground on southern slopes, most of the dead roots in the field previously mentioned were so much softened that they could be recognized. Many roots were much water-soaked in appearance, though fairly firm. Such roots when sectioned showed disorganization of tissue of greater or less extent although most of the cambium was apparently turgid and living. When turgid cells were situated along the margins of rifts they often gave a faint staining reaction characteristic of winter injury. The dead and injured tissue did not at this time show visible discoloration by which it could be recognized in the field.

On March 30, roots of alfalfa at Madison had begun to grow, and injured tissue in roots was usually so much discolored that it could be seen from examination of plants in the field. The condition of the root of a small seedling that barely survived its first winter is shown in Figure 6. Here only small islands of cells with the fascicular cambium at their center had survived, and new conducting elements to support the living buds above were beginning to mature. The islands of living cells were more or less surrounded with the material that takes the characteristic winter-injury stain.

By the end of April all the dead and injured tissue was visibly discolored, and sometimes a stain diffused from it into the uninjured tissue. In one field taproots completely black, almost like ink when cut across below the crown lost most of this color if soaked in water, and when sectioned showed a vigorous living cambium. After the plants had begun to grow vigorously the precise limits of injury were easily discovered by cutting the root across with a knife, though

several months might elapse before the outer phloem decayed sufficiently to produce the roughened appearance shown in Plate 1, B.

The characteristic appearance of cross sections of injured roots after growth has started vigorously is shown in Plate 2. In Plate 2, A, the injury is largely confined to the phloem, which is beginning to blacken. Black dots in the wood indicate groups of vessels that are showing the presence of injury by gum deposit and discoloration of a few surrounding parenchymatous cells. A greater degree of injury is shown in Plate 2, B. The death of tissue at the center of the root and along the wood rays had led to extensive cracking of the wood and opening of the center from the pressure of the vigorous new growth. Although the limits of the new growth can be seen clearly almost all the way around the periphery, a stain from the decaying tissue obscures the healthy tissue in one small sector. In spite of this injury, the growth of this plant seemed scarcely less vigorous than that of uninjured plants. At this stage the exact limits of the injury are best seen in stained microscopic sections. At all the small isolated places where cells seem to have been separated, the characteristic staining material has been deposited on the cell walls along the rift. So long as the plant lives it retains these evidences of injury—evidences that will usually reveal upon examination the year in which they occurred. Thus it is possible by the examination of a number of plants to discover the years in which any old field suffered especially severe injury. An instance in point may be cited. One of the disastrous winters for alfalfa in southern Wisconsin was that of 1921–22. Among the collections of wilt-infected plants from the southern part of the State made in 1925 and 1926 were two from fields that were sown in 1921 and that had therefore survived this winter. An examination of sections from these collections showed that almost every plant had in its first year's wood plugged vessels characteristic of winter injury as well as phloem injury when the phloem was present. It may be added here that the examination of other collections of wilt-infected plants from Kansas showed that while there was extensive winter injury in 1924–25 and 1925–26 in eastern Kansas, little of such injury originated in the two preceding years.

GROWTH RESPONSE OF INJURED PLANTS

The growth response of plants to climatic injury appears to be similar to their response to mechanical wounds. All of the parenchymatous tissue in which the larger part of the injury takes place seems to be able in varying degree to become a meristem capable of laying down a cork layer except perhaps the wood ray cells that have become considerably elongated axially. Injury in the phloem and in the central pithlike parenchyma meets the quickest response in the surrounding cells. In the phloem parenchymatous cells beneath those injured quickly begin to divide (fig. 4) and a new corky covering is produced like the original phelloderm. If the new cork arises in immature cells near the cambium it is usually unbroken and effectual. If it arises in mature phloem parenchyma it is usually interrupted by groups of fibers, which pursue a sinuous course through this tissue, and through these fibers decay traverses the cork layer into the underlying cells. These cells usually are able to divide rapidly enough

to prevent the decay from progressing far, but in so doing the outer bark is sometimes ruptured by the new growth beneath and new repairs must be made until the outer appearance of the root becomes very rough and irregular.

Healing in the central pith takes place in a similar manner. Small wounds in the first year's wood are usually healed quickly and effectually (fig. 10) if no fibers are involved. Groups of fibers cause difficulty here as in the phloem.

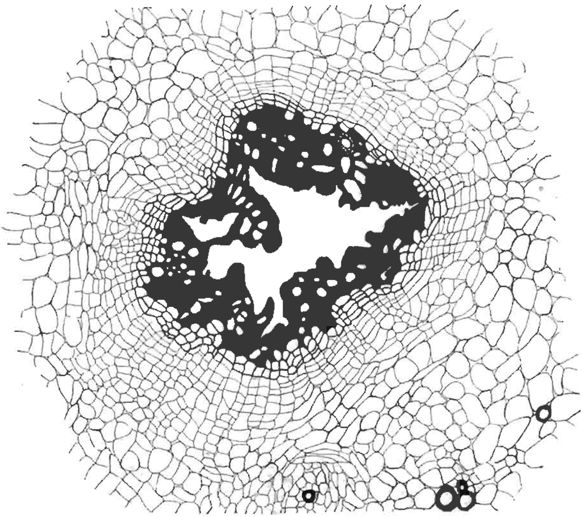
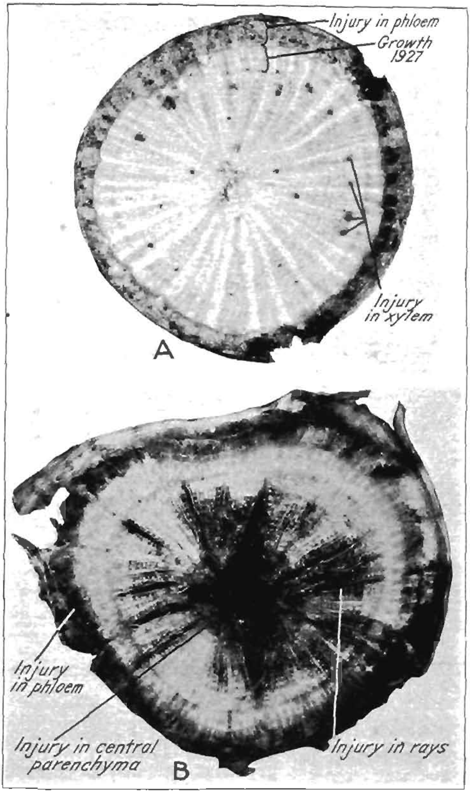


FIG. 10.—Small discolored spot at the center of a 6-year-old alfalfa plant of a hardy variety at Madison, Wis. This spot may have originated from injury in the central parenchyma during the first winter. It has been so well surrounded by the meristematic activity of parenchyma that further decay is prevented

Repair of injury along the wood rays that are completely split open takes place by division of smaller cells along the margin of the bundle which belong perhaps to the wood parenchyma. These cells are not very active in division, especially when they are a year or more old, and if they do divide extensively the mechanical push in a tangential direction that results may break the interfascicular cambium if it was previously intact at this point, or in any case may cause an increase in diameter of the root or swelling that is characteristic of badly damaged plants that have grown rapidly afterwards. Thus the swollen roots consist of a ring of isolated vascular bundles. In Figure 11 is shown a section of one of the segments of such a ring,



Taproots of alfalfa plants showing winter injury, collected in Guthrie County, Iowa, May 17, 1927, after considerable spring growth had been laid down; injury occurred in the second winter that the plant passed through; cross sections of upper parts:

A.—Slight injury occurring chiefly in the phloem.

B.—Severe injury occurring in the phloem, the central parenchyma, the rays, and the wood.

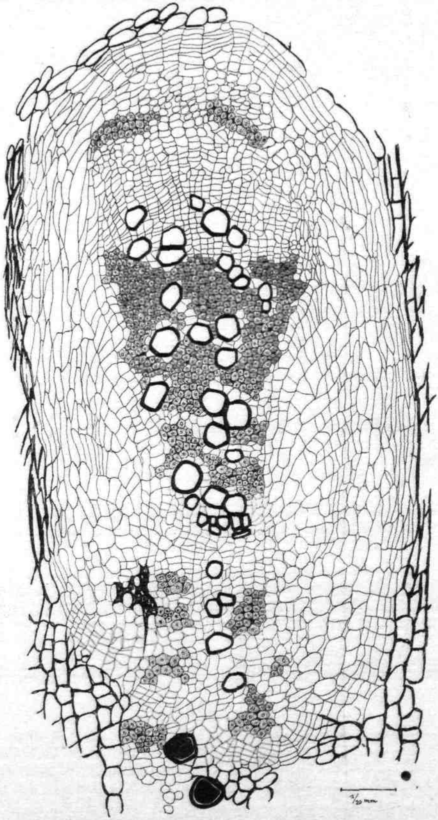


FIG. 11.—One vascular bundle from the upper part of the root of a hardy alfalfa plant whose bundles had been separated by killing of intervening parenchyma. The center of the root was hollow, surrounded by these separated bundles, each surrounded by meristematic tissue and separated from its neighbors by dead cells. Even though healed thus effectually, the plant was not thrifty

which may have been brought about by injury like that shown in Figure 6. Sometimes small local rifts in rays or elsewhere in parenchyma become filled with a gumlike material and no cell division follows.

In the mature wood there are two types of injury, the small brown spots arranged in circles (pl. 2, A) and the central injury. So far as the writer has been able to observe, the small spots are always surrounded sooner or later by thickened walls of the adjacent parenchyma or by the meristematic activity of such parenchyma so that decay does not spread to cause heart rot, as suggested by Weimer (*7*). Heart rot seems always to have its origin at the center of the plant. When the central tissue is killed during the first winter in the life of the plant, the necrotic cells can usually be completely separated from the healthy tissue by activity of the wood parenchyma, which is very abundant and is not interrupted by such large fiber masses as those in older growth. In older plants healing may be much more difficult. Not only do large fiber masses interrupt the parenchyma and by their slow decay continually extend the hollow center, but the mechanical action of swelling and shrinking of these woody masses with wetting and drying tends to tear apart the wood rays and expose new areas to fungous and bacterial invasion.

From the preceding it appears that the growth response to injury is conditioned by the character of the cells adjoining the injured tissue. If these cells are capable of prompt meristematic activity and the conditions surrounding the plant are favorable for growth, decay can not proceed far beyond the area originally killed. The loss of vascular tissue in the root does not appear to be a permanent handicap, and when new tissue has been produced abundantly, as shown in Plate 2, B, the plant continues growth with unabated vigor.

WINTER INJURY IN RELATION TO BACTERIAL WILT

During the last two years, in which winter injury has been seen in many fields in the central Mississippi Valley, much evidence has accumulated indicating that bacterial wilt (*3*) occurs in far greater abundance in such injured fields than in uninjured fields when sources of infection are approximately equal. Inasmuch as these parasitic bacteria require wounds through which to enter the plant, and since winter injury produces wounds apparently favorable for infection in the spring, when infection seems to take place most abundantly, it is highly probable that this injury is an important place of entry for the bacteria.

SUMMARY

A pathological condition of alfalfa roots and crowns caused apparently by adverse winter conditions has been described previously by Melchers and Weimer. The character of this injury is discussed in detail in this paper.

The development of the taproot and crown of alfalfa is traced and certain of their histological characteristics are described.

The injury in the plant tissue is found originating in characteristic locations in parenchyma of the phloem rays, in the central pithlike structure of the upper part of the taproot, and in the xylem.

In the spring of 1927 the injury appeared first as an apparent mechanical disorganization of tissue in plants taken from beneath an ice sheet. The healing of injuries in plants is traced through the spring.

Winter injury when severe appears not only to shorten the life of plants but to furnish a convenient point of entry for the parasitic bacterium (*Aplanobacter insidiosum* L. McC.) which causes bacterial wilt.

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A POSSIBLE RELATIONSHIP BETWEEN SOIL SALINITY AND STAND IN COTTON[1]

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INTRODUCTION

The problem of the factors influencing the germination of the seed and the establishment of the seedling is one of great interest in relation to succession in natural vegetation. It is of large economic importance not merely in the case of agricultural crop production but also in forest reproduction and in the reestablishment of native vegetation injured by overgrazing.

Relatively little seems to have been done in the application of statistical methods to the study of seedling stand[2] under field conditions.

The purpose of the present paper is to present the results of a first investigation of a possible relationship between soil salinity and seedling stand in Egyptian and upland cotton. The investigations on which this paper is based were conducted for the office of alkali and drought resistant crops, with the cooperation of the office of western irrigation agriculture and the seed laboratory of the Bureau of Plant Industry. They were carried out at the field station of the United States Department of Agriculture and the United States Indian Service at Sacaton, Ariz. While the results are not wholly consistent, they are so suggestive that it seems desirable to present them at this time because of their possible bearing on a number of important problems. Results of this kind are attained at such cost of labor that it is unlikely that another equally extensive series of observations can be secured and reduced to statistical constants at any time in the immediate future.

The specific problem undertaken was that of the possible correlation between the salinity of the soil and the number of seedlings established per unit area. The cotton plant is of particular interest for such investigations. Although cotton, like other crops, fails to produce a stand on extremely saline land, it is one of the most salt-resistant crops (*1, 2, 16, 17*).[3] Investigations have shown that the chloride (*14*) and sulphate (*11*) content of the tissue fluids of Egyptian and upland cotton is high, but that there is differential absorption for these anions (*12*). This differentiation holds not merely for the American-bred Pima Egyptian cotton as compared with several upland varieties, but also for several Egyptian varieties

[1] Received for publication July 2, 1928; issued October, 1928.

[2] The term "seedling stand," or "stand," rather than "germination rate" has been used because the census was made at the time when the plants had reached the stage of development at which thinning is usually done. It is possible that some of the seeds that had germinated might have died before this period.

[3] Reference is made by number (italic) to "Literature cited," p. 230.

grown from newly imported seed (*13*). Evidence has also been adduced to show that there is a more rapid accumulation of chlorides by the Egyptian type.

All these facts suggest that the relation of the cotton seedling to soil salinity may be quite different from that of many other agricultural plants. In a study of this relationship in the Egyptian and upland types of cotton, interest is heightened by the fact that the tissue-fluid properties of these cottons have been shown to be very closely correlated with the salt content of the substratum on which they are grown (*8*). Interest is further increased by the fact that the distribution of the stand per hill actually secured in cotton grown on the distinctly saline soils of the Southwest has been shown to differ significantly from a random distribution. This deviation from a theoretical or chance distribution consists in the production of an excess of hills with few or no seedlings and of an excess of hills with a large number of seedlings on fields planted with a uniform number of seeds per hill. The significance of these deviations has been tested by Pearson's χ^2 criterion (*10*) and the amount of deviation measured in terms of Pearson's equivalent probability correlation coefficient (*15*).

MATERIALS AND METHODS

The cultural methods will be described, and then the methods of statistical analysis of the experimental data will be given in necessary detail.

CULTURAL METHODS

The data analyzed were obtained from two cultures, one grown in 1922 (experiment 3/22), and the other in 1923 (experiment 1/23) on the rather saline alluvial soil of the Gila River Valley, Sacaton, Ariz. (*10, 15*).

The experiment of 1922, designated as experiment 3/22 in this paper and elsewhere, was conducted on the north halves of plots E 3-1, E 3-2, and E 3-3 of the United States Field Station at Sacaton, Ariz. All three plots were treated exactly alike and may be regarded as a unit experimental plot 79.5 feet in width and 200 feet in length, planted to 24 rows of cotton. Ten feet at each end were devoted to buffer cultures. Each row was divided into 18 subrows each 10 feet in length and planted to 10 hills of cotton.

The plantings of each of the three varieties, Pima Egyptian (P) and Meade (M) and Acala (A) upland cottons, were distributed over the field as follows:

	(1)			(2)			(3)				(6)		
(1)	A	P	M	A	P	M	A	P	M	. . .	A	P	M
(2)	A	P	M	A	P	M	A	P	M	. . .	A	P	M
.		.			.			.				.	
.		.			.			.				.	
.		.			.			.				.	
(24)	A	P	M	A	P	M	A	P	M	. . .	A	P	M

The numbers in parentheses at the top represent sections and the numbers in parentheses at the left side represent rows. Each of

these 10-foot sections planted to one variety may be designated as a plot. In consequence of this scheme of planting the three varieties are each distributed in 144 subrows or plots over the entire field.

If the field be heterogeneous with respect to soil salinity in the sense in which the term has been used in earlier investigations of the writer (*6*, *7*), and if the salinity of the soil has an influence on the germination rate or on the viability of the seedlings after germination, a correlation between the electrical resistance of the soil mass and the number of seedlings standing at a given time should be found.

Various phases of the problem of the physical heterogeneity of the field in the experiment of 1922 have already been treated. The results of these investigations show not merely that the field is heterogeneous with respect to soil properties as measured by the usual criterion (*6*), but also that this heterogeneity finds expression in the chloride content (*14*) and other physicochemical properties of the leaf-tissue fluids (*8*, *9*). They also show that the heterogeneity is itself a lawful phenomenon (*9*). They further show that the relationship between the properties of the soil solution and the physicochemical properties of the plant-tissue fluids may be measured directly in terms of the correlation coefficient or in terms of regression (*8*). In their bearing upon the present investigation these earlier results are of value in that they indicate the probable fruitfulness of the method of attack chosen.

In this experiment the soil samples were taken between rows 1 and 2, between 3 and 4, and so on, at three points approximately equally distributed over the 10-foot subrows devoted to each variety. This is exactly shown by a map published elsewhere (*9*). For the first triplet of the first four rows the arrangement is:

```
(1) A A A A A A A A A A P P P P P P P P P P M M M M M M M M M M . . .
         *     *     *     *     *     *     *     *     *     *
(2) A A A A A A A A A A P P P P P P P P P P M M M M M M M M M M . . .

(3) A A A A A A A A A A P P P P P P P P P P M M M M M M M M M M . . .
         *     *     *     *     *     *     *     *     *     *
(4) A A A A A A A A A A P P P P P P P P P P M M M M M M M M M M . . .
         .     .     .     .     .     .     .     .     .     .
         .     .     .     .     .     .     .     .     .     .
         .     .     .     .     .     .     .     .     .     .
(24) A A A A A A A A A A P P P P P P P P P P M M M M M M M M M M . . .
```

Here the letters A, P, M represent 10 individual plantings (hills) of each of the varieties shown in the scheme on page 214.

A combination of the three soil cores taken at the positions approximately indicated by the asterisks between each of the varieties of the two adjoining rows provided the soil samples necessary for the reading of the electrical conductivity of the saturated soil mass by means of the electric bridge described by Davis and Bryan (*3*). Determinations were reduced to the standard temperature of 60° F. by the table given by Davis and Bryan. Since rows 1 and 2 and subsequent pairs of rows are equally and but slightly separated from the series of borings made between them, the same soil sample may serve for the two rows.

Thus in this experiment there were 216 sets of soil conductivity determinations (72 for each variety), each comprising readings of four individual soil layers of 1-foot depth. The conductivities of these 72 sets of soil samples for each variety were weighted with the number of hills of the given variety in each two-row plot, which was 20 in each case. This makes a total of 1,440 hills, or a total of 72 plots weighted in a twofold manner, employed as a basis for the determination of correlations in each variety. Methods of weighting employed to eliminate the influence of wholly sterile hills will be indicated below.

In the experiment of 1923 (experiment 1/23) 16 rows on plots D 1–10 and D 1–11 of the United States field station, Sacaton, Ariz., were planted to alternate sections of Pima Egyptian and Lone Star upland cottons, and between each pair of these was planted 1 hill of hybrid cotton. Thus the order of planting was: 18 hills of Pima, 1 hill of hybrid, 18 hills of Lone Star, 1 hill of hybrid, 18 hills of Pima, etc. The 9 hills of Pima and the 9 hills of Lone Star cotton on either side of each hybrid plant may be considered as constituting an experimental unit for the purposes of the present study. There were 10 such units per row, which would therefore comprise $90 \times 16 =$ 1,440 hills of each variety. The hybrid plants are not considered here.

Soil samples were taken between rows 1 and 2, between 3 and 4, between 5 and 6, and so on, in the manner indicated on page 215 for the scheme for the 1922 experiment, except that in this case the soil samples were taken in the immediate neighborhood of the hybrid plants.

Thus 80 sets of conductivities are available for both varieties. These have been weighted with the number of hills of each variety, which was 18 for the sections of the two adjoining rows, compared with the soils of each boring, or with the number of sections of rows, which was 2, in determining the correlations between soil resistance and seedling stand. A total of 1,440 hills is therefore involved, or a total of 80 plots weighted in a twofold manner. Methods of weighting employed in dealing with sterile hills will be indicated below.

METHODS OF STATISTICAL ANALYSIS OF DATA

In determining correlations between soil resistance and number of seedlings per hill the resistances were grouped in classes of 25-ohm range in the case of experiment 3/22. In experiment 1/23 classes of 15-ohm range were practicable. In determining the average resistance of the upper 4 feet of soil, the original resistances were first averaged and then grouped in classes of the range indicated for the individual feet of the two experiments.

The methods of determining the statistical constants will now be considered.

Let s be the number of seedlings per hill in each of the n hills of the N plots for which records of soil resistance, R, and seedling number are available. The problem is merely to determine the correlation between soil resistance, as an approximate measure of soil salinity, and number of seedlings germinating and surviving to the time of record.

In all the experiments six seeds were planted per hill. The number of seedlings correlated may therefore vary from none to six. It has been shown elsewhere (*10, 15*) that there is reason for believing that special factors are involved in determining the complete absence of seedlings in the case of certain of the hills. It seems desirable, therefore, to consider the data in two classes, (1) those involving all hills; and (2) those involving hills in which at least one seedling was found at the time of thinning. Let n be the number of hills per plot and n' the number of hills per plot that produce at least one seedling. Let s denote the number of seedlings in the hills of the first class (none to six seedlings per hill) and s' the number of seedlings per hill in the second class (one to six seedlings per hill). The total number of hills involved in the correlations of the first class, r_{Rs}, will be Nn. The total number of hills in correlations of the second class, $r_{Rs'}$, will be $S(n')$, where S denotes summations for the several plots. In determining these correlations the soil resistance is weighted with the constant value of n in the first instance and with the variable value of n' in the second instance.

If the method of taking moments about 0 as origin (*4, 5*) be employed,

$$\overline{R}=S(nR)/Nn \quad \text{(1)}$$

$$\overline{R}'=S(n'R)/S(n') \quad \text{(2)}$$

$$\sigma^2_R=\frac{S(nR^2)}{Nn}-\left(\frac{S(nR)}{Nn}\right)^2 \quad \text{(3)}$$

$$\sigma'^2_R=\frac{S(n'R^2)}{S(n')}-\left(\frac{S(n'R)}{S(n')}\right)^2 \quad \text{(4)}$$

where the primes denote constants due to weighting with the number of hills producing one or more seedlings, n', rather than with the constant number of hills planted per plot, n, and the bars and σ's denote means and standard deviations, respectively.

Let Σ denote summation within individual plots and S denote summation from plot to plot throughout the experimental field. Then $\Sigma(s')=\Sigma(s)$, $\Sigma(s'^2)=\Sigma(s^2)$, and for the whole population

$$\overline{s}=S[\Sigma(s)]/Nn=S[\Sigma(s')]/Nn \quad \text{(5)}$$

$$\overline{s}'=S[\Sigma(s')]/S(n')=S[\Sigma(s)]/S(n') \quad \text{(6)}$$

$$\sigma^2_s=\frac{S[\Sigma(s^2)]}{Nn}-\left(\frac{S[\Sigma(s)]}{Nn}\right)^2=\frac{S[\Sigma(s'^2)]}{Nn}-\left(\frac{S[\Sigma(s')]}{Nn}\right)^2 \quad \text{(7)}$$

$$\sigma'^2_s=\frac{S[\Sigma(s'^2)]}{S(n')}-\left(\frac{S[\Sigma(s')]}{S(n')}\right)^2=\frac{S[\Sigma(s^2)]}{S(n')}-\left(\frac{S[\Sigma(s)]}{S(n')}\right) \quad \text{(8)}$$

The correlations are:

$$r_{Rs}=\frac{\dfrac{S[R\Sigma(s)]}{Nn}-\left(\dfrac{S(nR)}{Nn}\cdot\dfrac{S[\Sigma(s)]}{Nn}\right)}{\sigma_R\sigma_s}=\frac{\dfrac{S[R\Sigma(s')]}{Nn}-\left(\dfrac{S(nR)}{Nn}\cdot\dfrac{S[\Sigma(s')]}{Nn}\right)}{\sigma_R\sigma_s}\qquad(9)$$

$$r_{Rs}'=\frac{\dfrac{S[R\Sigma(s')]}{S(n')}-\left(\dfrac{S(n'R)}{S(n')}\cdot\dfrac{S[\Sigma(s')]}{S(n')}\right)}{\sigma'_R\sigma'_s}=\frac{\dfrac{S[R\Sigma(s)]}{S(n')}-\left(\dfrac{S(n'R)}{S(n')}\cdot\dfrac{S[\Sigma(s)]}{S(n')}\right)}{\sigma'_R\sigma'_s}\qquad(10)$$

While the record of the number of seedlings surviving in a single hill may furnish some information concerning the properties of the soil as a factor influencing stand, it can not be a very reliable measure because of its demonstrably high variability.

Another and presumably better measure is furnished by the average production of the plot, $\Sigma(s)/n=\Sigma(s')/n$, or by the total number of seedlings per plot, say for convenience of notation $P=\Sigma(s)=\Sigma(s')$.

The constants of P are

$$\overline{P}=S[\Sigma(s)]/N=S[\Sigma(s')]/N\qquad(11)$$

$$\sigma^2_P=S\{[\Sigma(s)]^2\}/N-\{S[\Sigma(s)]/N\}^2=S\{[\Sigma(s')]^2\}/N-\{S[\Sigma(s')]/N\}^2\qquad(12)$$

and

$$r_{RP}=\frac{S[R\Sigma(s)]/N-\overline{R}\overline{P}}{\sigma_R\sigma_P}=\frac{S[R\Sigma(s')]/N-\overline{R}\overline{P}}{\sigma_R\sigma_P}\qquad(13)$$

RESULTS

While a detailed discussion or description of the plots in biometric terms is unnecessary, it is desirable to show the mean values and the variabilities of soil resistance in the two experiments. These appear in Table 1. The constants here given are based on the actual number of soil samples analyzed. They are the constants used in determining the correlations between soil resistance and the number of seedlings per hill in the entire series, r_{Rs}, and between the soil resistance and the number of seedlings per plot, r_{RP}. The constants employed in the determination of the correlations for soil resistance and number of seedlings per hill in the selected series, which is limited to the hills that contained at least one seedling, are somewhat modified by weighting. These weighted values are not given, since all that is required for the purpose of the present discussion is a general idea of the order of magnitudes of the conductivities.

TABLE 1.—*Statistical constants for soil resistance at successive 1-foot depths, R_1, R_2, R_3, R_4, in plots at the United States Field Station, Sacaton, Ariz.*

Layers of soil	Mean	Standard deviation	Coefficient of variation
Experiment 3/22:			
Pima (Egyptian)—			
First foot, R_1	213.19±4.25	53.52±3.01	25.10
Second foot, R_2	200.00±7.89	99.30±5.58	49.65
Third foot, R_3	130.56±6.56	82.52±4.64	63.21
Fourth foot, R_4	100.35±4.27	53.76±3.02	53.58
First to fourth foot, R_{1-4}	160.76±5.15	64.79±3.64	40.30
Meade (upland)—			
First foot, R_1	211.80±4.20	52.86±2.97	24.96
Second foot, R_2	196.18±7.47	93.97±5.28	47.90
Third foot, R_3	123.61±5.51	69.33±3.90	56.09
Fourth foot, R_4	91.67±3.99	50.17±2.82	54.73
First to fourth foot, R_{1-4}	153.82±4.74	59.61±3.35	38.75
Acala (upland)—			
First foot, R_1	204.17±4.02	50.52±2.84	24.74
Second foot, R_2	195.14±7.65	96.25±5.41	49.32
Third foot, R_3	127.78±6.55	82.45±4.63	64.52
Fourth foot, R_4	93.75±3.71	46.72±2.63	49.84
First to fourth foot, R_{1-4}	154.86±4.96	62.45±3.51	40.33
Experiment 1/23:			
Pima (Egyptian) and Lone Star (upland)—			
First foot, R_1	157.88± .89	49.97± .63	31.65
Second foot, R_2	164.81±1.13	63.08± .80	38.27
Third foot, R_3	145.12±1.08	61.06± .77	42.07
Fourth foot, R_4	147.75±1.15	64.52± .81	43.67
First to fourth foot, R_{1-4}	155.06± .95	53.61± .67	34.57

The soil resistances are low, being of the general order of magnitude of 150 ohms at 60° F., thus indicating relatively high salinity. No attempt has been made to express these salinities in terms of the actual salt-content scale, since for purposes of comparison the original resistances probably represent a better measure of the soil solution than would any values computed from them.

A conspicuous feature of these results is that the general order of magnitude of the soil resistances in the two series is about the same. Salinities at the surface are somewhat higher in the experiment of 1923 than in that of 1922. Another conspicuous feature is the high variability of soil resistance from plot to plot. This variability is measured by standard deviations ranging from about 47 to about 99 ohms and by coefficients of variation ranging from about 25 to about 65 per cent. While these high variabilities are advantageous for the purposes of this investigation, since they show that the plots on which the experiments were carried out are highly heterogeneous, they also show that we are dealing with a phenomenon so variable that results of the highest precision can not be expected.

The means, standard deviations, and coefficients of variation of number of seedlings per hill in all hills (whole series), s; of seedlings per hill in the series selected to contain only hills producing at least one seedling (selected series), s'; and of number of seedlings per plot, P, appear in Table 2.

TABLE 2.—*Statistical constants for number of seedlings per hill and per plot in Pima, Meade, Acala, and Lone Star cottons as grown at the United States Field Station, Sacaton, Ariz., in 1922 and 1923*

Experiment and variety	Whole series (per hill)			Selected series (per hill)			Seedlings per plot		
	Mean	Standard deviation	Coefficient of variation	Mean	Standard deviation	Coefficient of variation	Mean	Standard deviation	Coefficient of variation
Experiment 3/22:									
Pima (Egyptian)	1.98±0.03	1.94±0.02	98.19	3.08±0.03	1.57±0.02	50.88	19.76±0.61	10.82±0.43	54.75
Meade (upland)	1.31± .03	1.55± .02	117.87	2.38± .03	1.34± .02	56.54	13.12± .43	7.60± .30	57.98
Acala (upland)	2.08± .03	1.80± .02	86.61	2.91± .03	1.45± .02	49.94	20.78± .39	9.82± .39	47.27
Experiment 1/23:									
Pima (Egyptain)	1.17± .03	1.73± .02	148.59	2.85± .04	1.60± .03	56.07	10.51± .43	8.15± .31	77.55
Lone Star (upland)	1.05± .03	1.61± .02	152.96	2.70± .04	1.48± .03	54.87	9.45± .40	7.42± .28	78.50

The most conspicuous feature of this table is the low mean number of seedlings per hill. Six seeds were planted per hill in each case, but when all hills are included the average production is less than 1.5 seedlings per hill in three of the series and less than 2.25 seedlings per hill in the other two series. This is due primarily to the fact that a large number of hills produce no seedlings at all. This has been shown in the diagrams of an earlier paper (*10*). Even when hills producing no seedlings at all are excluded, as in the selected series, the average number of seedlings per hill reaches three in only one of the five series.

Thus it appears that in these experiments the stand produced was very poor. This is particularly true of experiment 1/23. It is quite possible that had all the conditions of growth been such as to produce on an average a more complete stand the correlation coefficients would have shown a more material magnitude and greater uniformity. Neither large nor uniform correlations can be expected with such high coefficients of variation as are characteristic of these experiments.

The variabilities, both absolute and relative, are of special interest. The coefficients of variation for number of seedlings per hill in the series in which hills producing no seedlings were included ranges from 87 to 153. When the calculations are based on the selected series, the standard deviations range from 50 to 56 per cent of the mean. When the variabilities of number of seedlings per plot are dealt with, the standard deviations range from 47 to 79 per cent of the average stand of these plots. It will be quite clear from these high coefficients that we are here dealing with a phenomenon so variable that smooth results can hardly be expected.

The correlations between soil resistance and seedling stand for the experiment of 1922, appear in Table 3, which shows the correlation between the soil resistance of the first to fourth foot, R_1 to R_4, and the number of seedlings germinating. The averages of these correlations are also given. These averages are not provided with probable errors, since it is not quite clear how these should be calculated. As a further measure of the relationship between the properties of the soil and the number of seedlings, the correlation between the average resistance of the soil of the first 4 feet, R_{1-4}, and the number of seedlings is also given. While there may be some question from both the physical and the biological standpoint as to the legitimacy of averaging

the resistances of different layers of soil as a more general measure of the salinity of the soil mass as a whole, earlier studies (*8*) have shown that a closer correlation between soil properties and plant characteristics are thus secured.

The second column of Table 3 shows the relationship between soil resistance and the number of seedlings per hill (r_{Rs}) when all hills are included, irrespective of whether they produced seedlings. The fourth column shows the correlation between soil resistance and the number of seedlings per hill ($r_{Rs'}$) for hills that contain at least one seedling. The sixth column shows the difference between these two coefficients. The probable errors of these differences have been determined without regard to possible correlation between the two correlations. They are, therefore, probably too large. The eighth column shows the correlations between soil resistance and the total number of seedlings per plot (r_{RP}).

TABLE 3.—*Correlation coefficients measuring the relationship between soil salinity in terms of the electrical resistance of the soil mass and seedling stand in Egyptian and upland cottons grown at the United States Field Station, Sacaton, Ariz., in 1922*

Depth of sample	Correlation between soil resistance and number of seedlings per hill (whole series) r_{Rs}		Correlation between soil resistance and number of seedlings per hill (selected series) $r_{Rs'}$		Difference between correlation in whole series and selected series $r_{Rs}-r_{Rs'}$		Correlation between soil resistance and number of seedlings per plot $r_{RP'}$	
	$r \pm E_r$	r/E_r	$r \pm E_r$	r/E_r	$Diff. \pm E_{diff.}$	$Diff./E_{diff.}$	$r \pm E_r$	r/E_r
Pima (Egyptian):								
First foot, R_1	−0.2048 ± .0170	12.03	−0.0975 ± .0220	4.44	−0.1073 ± .0278	3.86	−0.3490 ± .0494	7.07
Second foot, R_2	− .2002 ± .0171	11.74	− .0847 ± .0220	3.84	− .1155 ± .0279	4.14	− .3476 ± .0494	7.03
Third foot, R_3	− .1617 ± .0173	9.34	− .0690 ± .0221	3.12	− .0927 ± .0281	3.30	− .2812 ± .0518	5.43
Fourth foot, R_4	− .1440 ± .0174	8.27	− .0813 ± .0221	3.69	− .0627 ± .0281	2.23	− .2494 ± .0527	4.73
Average correlation	− .1777	......	− .0831	......	......	......	− .3068	......
First to fourth foot, R_{1-4}	− .2062 ± .0170	12.12	− .1020 ± .0220	4.64	− .1042 ± .0278	3.75	− .3563 ± .0491	7.26
Meade (upland):								
First foot, R_1	− .1788 ± .0172	10.40	− .1084 ± .0236	4.59	− .0704 ± .0292	2.41	− .3006 ± .0511	5.88
Second foot, R_2	− .1506 ± .0174	8.67	− .0955 ± .0237	4.03	− .0551 ± .0294	1.87	− .2802 ± .0518	5.41
Third foot, R_3	− .1198 ± .0175	6.84	− .0750 ± .0238	3.15	− .0448 ± .0295	1.52	− .2213 ± .0535	4.14
Fourth foot, R_4	− .0999 ± .0176	5.68	− .0742 ± .0238	3.12	− .0257 ± .0296	.87	− .1771 ± .0544	3.25
Average correlation	− .1373	......	− .0883	......	......	......	− .2448	......
First to fourth foot, R_{1-4}	− .1531 ± .0174	8.82	− .1067 ± .0236	4.51	− .0464 ± .0293	1.58	− .2756 ± .0519	5.30
Acala (upland):								
First foot, R_1	− .1577 ± .0173	9.10	− .0559 ± .0210	2.67	− .1018 ± .0272	3.74	− .3448 ± .0495	6.96
Second foot, R_2	− .1048 ± .0176	5.96	− .0123 ± .0210	.58	− .0925 ± .0274	3.38	− .2058 ± .0538	3.82
Third foot, R_3	− .1147 ± .0175	6.54	− .0315 ± .0210	1.50	− .0832 ± .0273	3.05	− .2268 ± .0533	4.25
Fourth foot, R_4	− .1205 ± .0175	6.88	− .0634 ± .0210	3.03	− .0571 ± .0273	2.09	− .2408 ± .0530	4.55
Average correlation	− .1244	......	− .0408	......	......	......	− .2546	......
First to fourth foot, R_{1-4}	− .1526 ± .0174	8.79	− .0553 ± .0210	2.63	− .0973 ± .0273	3.56	− .3061 ± .0509	6.01

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A glance at Table 3 shows that the coefficients are negative in sign throughout. Since soil salinity is measured in terms of electrical resistance in ohms, the negative values of these coefficients indicate that in this experiment higher soil salinities are accompanied by higher seedling stands.

The first measure of interrelationship (the correlations between soil resistances and number of seedlings in all hills, r_{Rs}) shows that the coefficients are from 5.68 to 12.12 times as large as their probable errors. There can, therefore, be no question that in this series of determinations the more saline portions of the field, which are characterized by lower electrical resistances, produce a larger seedling stand. The average correlations are of the order −0.124 (Acala upland) to −0.178 (Pima Egyptian). The correlations for the average resistance of the first to fourth foot are larger than the average correlations, but not larger than all of the individual constants for the several soil layers.

Correlations between soil resistance and the number of seedlings per hill in the series comprising only hills that produced at least one seedling ($r_{Rs'}$) are negative throughout, but are smaller in comparison with their probable errors than are the preceding constants. Their ratios to their probable errors, which have been calculated by the usual formula, range from 0.58 to 4.64.

The algebraic differences between these correlations and those based on all of the hills (including sterile hills) are negative throughout. They show that the correlations are more negative when the vacant hills are included than when the coefficients are based on a selected series of hills each including at least one seedling. It may be noted, however, that inasmuch as all signs are negative, the algebraic differences will differ only in sign from the numerical differences in the same direction, which are consistently positive. The significance of the numerical values of the differences therefore offers no complication.

The fact that the coefficients as determined by both methods are consistently negative would seem to indicate that under the conditions of this experiment the salinity of the soil has such a definite influence upon the seedling stand that a more perfect stand is produced on sections of the field having higher soil salinity. This conclusion is strengthened by the fact that the inclusion of the hills which produced no seedlings increases the magnitude of the correlation.

The correlations between the resistance of the soil mass and the total number of seedlings per plot are in every instance conspicuously higher than either of the preceding values. They range from −0.177 to −0.356, with average values of −0.307 for Pima Egyptian, −0.245 for Meade upland, and −0.255 for Acala upland cotton. These averages are based on the coefficients determined from the upper 4 feet of soil, and do not include the coefficients based on the average resistance of these 4 feet. No probable errors are given for these averages, since only four cases are involved.

The correlations for the average resistance of the upper 4 feet of soil and the seedling stand produced are of the same order of magnitude as the average of the correlations for the individual soil layers and seedling stand. These coefficients are from 3.25 to 7.26 times as large as their probable errors. Thus there can be no question concerning their individual trustworthiness.

It is interesting, and possibly significant, that in general the correlations between the resistances of the upper foot or the upper 2 feet of soil and seedling stand are higher than those for the lower layers of soil. This is exactly what might be expected if the salinity of the soil directly influenced seedling stand. It is important to note, however, that the soil samples were taken near the end of the season, after the tissue-fluid determinations with which they have also been correlated (*8*) were made, and not at the exact time that seedling stand was being established. Doubtless some vertical movements of salts occurs with the march of the season.

Since the values of the correlation coefficients are low, it is reasonable to expect considerable irregularity in the distribution of the empirical means about the regression lines. Nevertheless, it has seemed desirable to determine the straight-line equations showing the relationship between number of seedlings per hill and soil salinity in the total series and in the selected series.

The straight-line equations showing the regression of the number of seedlings per hill on the soil resistance for the three varieties of cotton appear in Table 4. The lines for Pima Egyptian cotton are represented in Figure 1, those for Meade upland cotton in Figure 2, and those for Acala upland cotton in Figure 3.

TABLE 4.—*Regression of number of seedlings per hill on soil resistance, R, for Pima, Meade, and Acala cottons as grown at the United States Field Station, Sacaton, Ariz., in 1922*

Layers of soil	Regression for Pima (Egyptian)	Regression for Meade (upland)	Regression for Acala (upland)
First foot, R_1	$s = 3.5567 - 0.0074\ R_1$ $s' = 3.6611 - .0028\ R_1$	$s = 2.4198 - 0.0052\ R_1$ $s' = 2.9174 - .0027\ R_1$	$s = 3.2255 - 0.0056\ R_1$ $s' = 3.2241 - .0016\ R_1$
Second foot, R_2	$s = 2.7570 - .0039\ R_2$ $s' = 3.3449 - .0014\ R_2$	$s = 1.7979 - .0025\ R_2$ $s' = 2.6385 - .0014\ R_2$	$s = 2.4609 - .0020\ R_2$ $s' = 2.9477 - .0002\ R_2$
Third foot, R_3	$s = 2.4710 - .0038\ R_3$ $s' = 3.2561 - .0014\ R_3$	$s = 1.6422 - .0027\ R_3$ $s' = 2.5508 - .0015\ R_3$	$s = 2.3984 - .0025\ R_3$ $s' = 2.9823 - .0006\ R_3$
Fourth foot, R_4	$s = 2.4960 - .0052\ R_4$ $s' = 3.3219 - .0025\ R_4$	$s = 1.5940 - .0031\ R_4$ $s' = 2.5648 - .0022\ R_4$	$s = 2.5137 - .0046\ R_4$ $s' = 3.0968 - .0020\ R_4$
First to fourth foot, R_{1-4}	$s = 2.9672 - .0062\ R_{1-4}$ $s' = 3.4799 - .0026\ R_{1-4}$	$s = 1.9225 - .0040\ R_{1-4}$ $s' = 2.7404 - .0025\ R_{1-4}$	$s = 2.7596 - .0044\ R_{1-4}$ $s' = 3.1088 - .0013\ R_{1-4}$

In these figures the lower lines, with empirical means represented by circles, show the regression of number of seedlings per hill on soil resistence in the complete series. The upper lines, with empirical means represented by solid dots, show the regression of the number of seedlings per hill on soil resistance in the selected series. Since the mean number of seedlings per hill in the selected series is higher than that in the whole series (because of the omission of hills with no seedlings) the lines for the selected series lie above those for the whole series.

All the regression lines show only slight slopes, as is to be expected from the low values of the correlation coefficients. While the empirical means are distributed with considerable irregularity about the lines, there can be no question that a straight-line equation represents the results as well as they can be graduated by any one equation. This result lends confidence to the writer's conclusion concerning the reality of the existence of a negative correlation between soil resistance and seedling stand in the 1922 experiment, on which the conclusions of the present paper are primarily based.

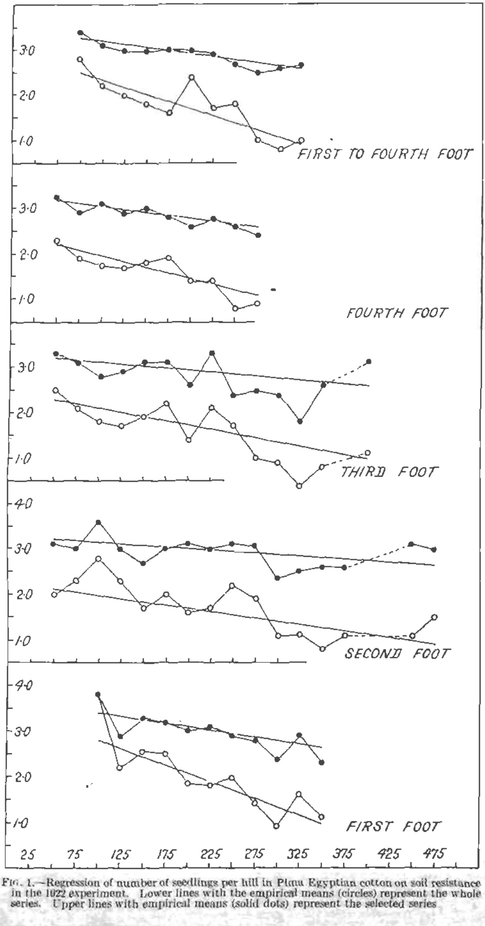

FIG. 1.—Regression of number of seedlings per hill in Pima Egyptian cotton on soil resistance in the 1922 experiment. Lower lines with the empirical means (circles) represent the whole series. Upper lines with empirical means (solid dots) represent the selected series

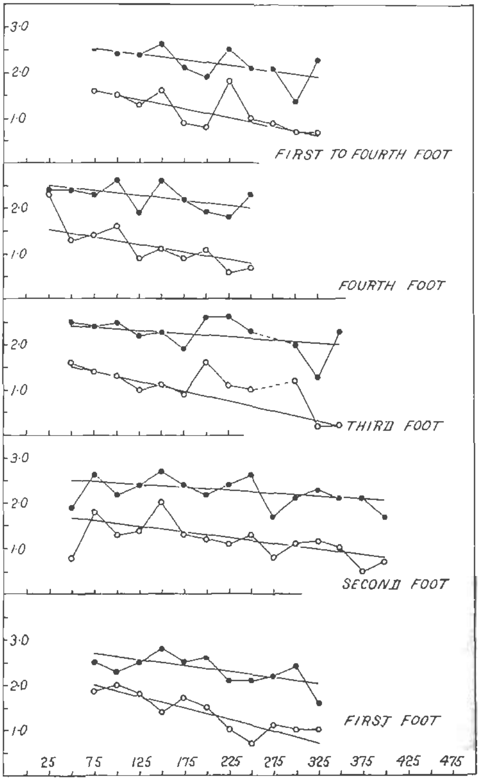

FIG. 2.—Regression of number of seedlings per hill in Meade upland cotton on soil resistance in the 1922 experiment. Lower lines with the empirical means (circles) represent the whole series. Upper lines with empirical means (solid dots) represent the selected series

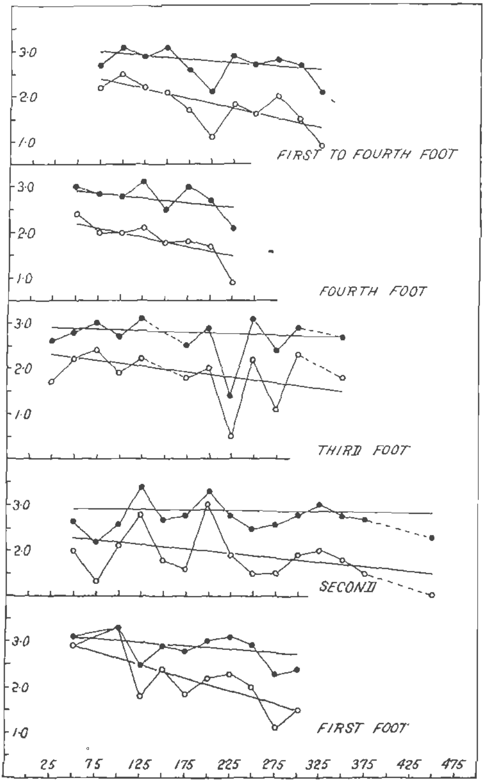

FIG. 3.—Regression of number of seedlings per hill in Acala upland cotton on soil resistance in the 1922 experiment. Lower lines with the empirical means (circles) represent the whole series. Upper lines with empirical means (solid dots) represent the selected series

The correlation coefficients showing the relationship between soil resistance and number of seedlings per hill and per plot in the series of determinations of the 1923 experiment appear in Table 5, which is comparable with Table 3.

TABLE 5.—*Correlation coefficients measuring the relationship between soil salinity in terms of the electrical resistance of the soil mass and seedling stand in Egyptian and upland cottons grown at the United States Field Station, Sacaton, Ariz., in 1923*

Depth of sample	Correlation between soil resistance and number of seedlings per hill (whole series) r_{Rs}		Correlation between soil resistance and number of seedlings per hill (selected series) $r_{Rs'}$		Difference between correlation in whole series and selected series $r_{Rs}-r_{Rs'}$		Correlation between soil resistance and number of seedlings per plot r_{RP}	
	$r\pm E_r$	r/E_r	$r\pm E_r$	r/E_r	$Diff.\pm E_{diff.}$	$Diff./E_{diff.}$	$r\pm E_r$	r/E_r
Pima (Egyptian):								
First foot, R_1	+0.0741 ±.0177	4.19	+0.0538 ±.0277	1.94	+0.0203 ±.0329	0.62	+0.1419 ±.0522	2.72
Second foot, R_2	−.0318 ±.0179	1.78	−.0220 ±.0278	.79	−.0098 ±.0331	.30	−.0614 ±.0535	1.15
Third foot, R_3	−.0572 ±.0177	3.23	−.0416 ±.0277	1.50	−.0156 ±.0335	.46	−.1096 ±.0527	2.08
Fourth foot, R_4	−.0587 ±.0177	3.32	−.0321 ±.0277	1.16	−.0266 ±.0329	.81	−.1125 ±.0526	2.14
Average correlation	−.0184		−.0105				−.0354	
First to fourth foot, R_{1-4}	−.0178 ±.0178	1.00	−.0141 ±.0278	.51	−.0037 ±.0330	.11	−.0342 ±.0533	.64
Lone Star (upland):								
First foot, R_1	+.0204 ±.0178	1.15	+.0661 ±.0284	2.33	−.0457 ±.0355	1.36	+.0398 ±.0532	.75
Second foot, R_2	−.0723 ±.0178	4.06	+.0770 ±.0283	2.72	−.1493 ±.0334	4.47	−.1420 ±.0526	2.70
Third foot, R_3	+.0076 ±.0178	.43	+.1487 ±.0278	5.34	−.1411 ±.0330	4.28	+.0148 ±.0533	.28
Fourth foot, R_4	−.0300 ±.0178	1.68	+.0689 ±.0283	2.43	−.0989 ±.0334	2.96	−.0584 ±.0531	1.10
Average correlation	−.0186		+.0902				−.0364	
First to fourth foot, R_{1-4}	−.0161 ±.0178	.91	+.1089 ±.0281	3.87	−.1250 ±.0333	3.75	−.0314 ±.0533	.59

The coefficients are low and extremely irregular in magnitude. In part they are positive and in part negative in sign. In general they can not be considered significant in comparison with their probable errors.

Considering first the correlation between the soil resistance and number of seedlings per hill in the whole series, r_{Rs}, we note that for Pima Egyptian cotton four of the correlations are negative and one is positive. For Lone Star upland cotton three of the coefficients are negative and two are positive. One of the positive coefficients (that for the first foot in Pima Egyptian cotton) may be significant in comparison with its probable error. The other two are certainly insignificant. The averages are slightly negative for both Pima Egyptian and Lone Star upland cottons.

The correlations between soil resistance and number of seedlings per hill in the series selected to contain only hills with at least one seedling are slightly positive in the Lone Star series but are negative in four of the five determinations for Pima Egyptian cotton. In this case the significance of the differences between the two series of correlations offers some complication. The differences in the table are algebraic and are consistently negative with the exception of that for

the first foot in the Pima Egyptian series. The numerical differences in the same direction ($r_{Rs} - r_{Rs}'$) are consistently positive for Pima and consistently negative for Lone Star.

The correlation between soil resistance and seedlings per plot are negative in 7 of the 10 cases. They are, however, low in magnitude.

The diversity of signs and the low numerical values of the correlation coefficients in this experiment show that no final conclusions can be drawn from it. There is some slight support for a positive correlation between soil salinity and seedling stand, as was so clearly indicated by the results of experiment 3/22, but these evidences are so slight as to be of little value.

The significance of these results will be discussed below.

DISCUSSION OF RESULTS

The results of the foregoing experiments are inconsistent in that the first, that of 1922, indicates a low but consistent and statistically significant relationship between the salinity of the soil and seedling stand in the case of Pima Egyptian, Meade upland, and Acala upland cottons, whereas the second, that of 1923, furnishes no conclusive evidence for the existence of such a relationship.

This inconsistency of results might at first thought seem to throw serious doubt upon the existence of any relationship between soil salinity and seedling stand. It unquestionably shows that final conclusions must await further investigations. It is unfortunate that the evidence of further series can not be presented immediately. Such experiments are, however, exceedingly laborious. It is unlikely that they can be completed in the near future. It seems proper, therefore, to present the constants derived from these two series, to suggest reasons for the inconsistency of the findings, and to offer possible interpretations of the results.

First of all, it must be noted that we are here dealing with highly variable characters. Seedling stand is on an average low in both of the available experiments and is presumably influenced by a number of factors. High correlations can not, therefore, be expected without the closest possible control of all conditions.

The results of the first experiment are wholly consistent throughout. If this were the only experiment available the existence of a definite relationship between soil salinity and seedling stand would be considered fully demonstrated. It is the belief of the writer that the inconclusive results of the second experiment are due primarily to its being technically less reliable than the first, and that it should be essentially disregarded in the drawing of conclusions. It has been included here merely because it is improper to suppress data that fail to support the conclusions drawn.

The 1922 experiment was carried out with the greatest care and in the greatest detail with a view to securing soils in immediate association with the plants. The soil borings were uniformly distributed over each of the 10-foot plots so that the soils were sampled very close to the seedlings, although at a date later than that at which the seedling records were made. In the 1923 experiment the soil samples were taken between the rows on either side of a group of hybrid plants that occupied a position between the upland and Egyptian plots. Thus the soil samples are unquestionably less representative of either

of the two varieties with which they are correlated than is the case in the 1922 experiment.

The question as to why the soil samples were not so accurately distributed with reference to the Pima Egyptian and Lone Star upland plants of the 1923 experiment as with reference to the Pima Egyptian and Meade and Acala upland plants of the 1922 experiment is pertinent. The answer is twofold: First, the 1923 experiment was planned primarily for a comparison of hybrid plants with the parent types lying immediately on either side of the hybrid group. It was not planned specifically with a view to determining the correlations between each of the parental types individually and the soil resistance of the immediate substratum on which the seedlings were produced. Second, the 1923 experimental work was carried out far in advance of the completion of the statistical analysis of the data secured in 1922. At the time the samples were taken in 1923 there was no reason to suspect that the relationship between the concentration of the soil solution and the characteristics of the plants would be so definite as it was subsequently demonstrated to be. There was no reason for believing that under field conditions such high correlations could be demonstrated as those shown in the investigation of the relationship between the concentration of the soil solution and the physicochemical properties of the plant-tissue fluids as was subsequently demonstrated on the basis of the experimental results of 1922 (*8*).

Thus the soil sampling of 1923 was carried out merely to obtain some general measure of the soil heterogeneity of this field and its relation to the plant. In consequence the records do not, in the opinion of the writer, represent as accurate or as valuable data for the purposes of this investigation as do those of 1922.

On a priori grounds the correlation between seedling stand and soil salinity under field conditions suitable for crop production would be expected to be low. Upon laying the correlations between soil resistance and seedling stand beside those for soil resistance and tissue-fluid properties as measured in 1922, we find that in that experiment the correlations for seedling stand are very small as compared with those for tissue-fluid properties. Under these conditions it is reasonable to suppose that a relationship between seedling stand and soil salinity would be demonstrable only under ideal technical conditions. Evidence that the conditions of 1923 were not ideal for the purpose of the phases of the investigation here under consideration is furnished by the fact that unpublished correlations between soil resistance and tissue-fluid properties in the 1923 experiment are lower than those in the 1922 experiment.

In view of the foregoing facts it seems quite probable that the failure to obtain correlations between soil resistance and seedling stand in 1923 comparable to those secured in 1922 may have been due to the experimental data being less suitable for the purposes of the investigation.

This comparison of results obtained in the two series is not without value in indicating the great care that is necessary in the execution of the technical phases of investigations of this kind.

The physical and physiological explanation of these results must be a subject for further investigation. It is probable that a factor of great importance in determining seedling stand in cotton may be the texture of the soil mass overlying the planted seeds. It is possible

that in the more saline soils the seedling encounters less difficulty in breaking through the soil layer than in the less saline soils. It is also possible that plants grown under the more saline conditions are less subject to the attack of some diseases. Both of these factors must be further investigated. Peculiarities of the frequency distributions of the numbers of seedlings produced per hill (*10, 15*) indicate that some such factors must be operative.

SUMMARY

The present investigation has dealt with the problem of the relationship between the concentration of the soil solution and the seedling stand produced in cultures of Pima Egyptian and of Meade, Acala, and Lone Star upland cotton on heterogeneous experimental fields.

Soil salinity was measured in terms of the electrical resistance of the saturated soil mass in the standard soil bridge cup. Seedling stand was recorded in terms of number of seedlings produced per hill in hills planted with a uniform number of seeds (six per hill), in terms of numbers of seedlings per hill in hills producing at least one seedling, and in terms of number of seedlings per plot, each plot comprising an equal number of hills.

The data for an experiment conducted in 1922, which was admirably designed to throw light on this problem, indicate that there is a significant negative correlation between soil resistance and seedling stand. Since soil resistance is measured in ohms, and therefore increases with diminishing salt content, this negative correlation indicates that under the range of soil salinities and other conditions of the experiment better stands are produced on more saline soils.

These relationships should not be expected to hold if cotton were planted on soils of indefinitely higher salt content. They may be typical of soils having the degree of salinity here involved.

The results of an experiment made in 1923 do not fully confirm those of the 1922 experiment. It is the belief of the writer that the 1923 experiment was technically inadequate for an investigation of this kind. The results are included merely to avoid bias in the presentation of data.

Assuming that under certain ranges of field conditions there is a positive correlation between the concentration of the soil solution and the seedling stand of cotton (or, as here expressed, a negative correlation between the electrical resistance of the soil solution as measured in the soil and seedling stand), further problems as to the physical, chemical, physiological and perhaps pathological causes of this relationship are presented for solution.

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A SURVEY OF THE RESISTANCE OF SUBSPECIES OF BRASSICA OLERACEA TO YELLOWS (FUSARIUM CONGLUTINANS)[1]

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INTRODUCTION

The wild cabbage (*Brassica oleracea* L.) is a native of the Old World, where it is found on the sea cliffs of western and southern Europe. From this leafy biennial or its progenitor have been derived presumably the cabbage (*B. oleracea* var. *capitata* L.), kale and collard (*B. oleracea* var. *acephala* DC.), cauliflower and broccoli (*B. oleracea* var. *botrytis* L.), Brussels sprouts (*B. oleracea* var. *gemmifera* DC.), and kohl-rabi (*B. oleracea* var. *caulo-rapa* DC.).[2] The organism (*Fusarium conglutinans* Woll.) causing yellows of this group of plants is apparently native to America and so far has not been reported from the Old World. Though first described as a disease of cabbage, it has been found on other forms of *B. oleracea*, and the data presented herewith show that the wild form and nearly all the varieties of the cultivated subspecies tested are more or less susceptible. Two reports of its occurrence on other species of Brassica have been made. According to Melhus,[3] Gilman found it upon the Chinese cabbage (*B. pekinensis* Rupr.) and Gregory reported it upon turnip (*B. rapa* L.).[4] The writers have never found it on the last two species, although they have included them in trials on infested soils where the disease developed abundantly on susceptible forms. The varieties of cabbage and its related forms have been developed largely in Europe out of contact with yellows and where natural or artificial selection for resistance through exposure to the disease could not have occurred.

The successful control of cabbage yellows has been based upon the fact that in every commercial variety so far tested at least a few individuals are highly resistant under field conditions. The selection of highly resistant strains from such individuals of several standard varieties has already been described.[5,6] Although none of

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the varieties popular in America seems to possess sufficient resistance in itself to be of commercial value for use on yellows-infested soil, no exhaustive study has been made of the degree of natural resistance, especially of varieties used commonly in Europe but seldom in America. The fact that Jones and Gilman [7] found two varieties, the Volga or Stonehead and the Houser, that possessed a marked degree of resistance leads one to expect that other such varieties might be found.

No previous study of the varietal differences in resistance among forms other than cabbage has been made. In connection with the program of selection of yellows-resistant varieties of cabbage it has been a matter of scientific and practical value to give some attention to the relative resistance not only among a larger number of cabbage varieties but also among the varieties of cabbage relatives. This paper is a report of such investigations, which have been under way since 1924.

METHODS

The method of testing was similar to that already described in connection with the trials of cabbage strains selected for resistence.[8] The test plot of soil, which is located in eastern Kenosha County, Wis., has become, through repeated cropping with cabbage, as uniformly and thoroughly infested with the yellows organism as possible under natural conditions. The seed was sown upon yellows-free soil about May 15, and the plants were transplanted to the infested soil during the first week of July. They were inspected several times during the season, and each plant that showed any sign of yellows was marked permanently with a bamboo stake. The symptoms of the disease on the cabbage relatives are so similar to those on cabbage that special description here seems unnecessary. As in cabbage, the severity of attack commonly varies among individuals of a given variety. Part of this variation may be due to environmental influences or variation in distribution of the pathogene, and part to hereditary differences, but these points can be settled only by further study. Some plants are killed rapidly; others are decidedly stunted, but not killed; others show very slight injury; and still others show traces of yellows in midseason on lower leaves that soon drop off, but mature with no apparent setbacks. From the practical standpoint one is interested in the total damage done by the disease, which obviously is not always truly represented when only the percentage of total plants showing yellows is given. At the end of the season, therefore, the affected plants were divided into two lots: (1) Those killed or severely injured, and (2) those showing only slight injury or apparently complete recovery.

The number of plants used for the test of a given variety was in some cases less than 25. This number may appear too small to compensate for variations in soil infestation. Larger trials and numerous replications are of course desirable, but time and space did not permit more extensive planting when so many lots were to be tested. Repeated use of the soil in question for similar purposes has given such consistent results that confidence in the reasonable accuracy of the method is sustained. Further weight is given the results by the repetition of many of the trials during two successive seasons.

[7] Jones, L. R., and Gilman, J. C. Op. cit.
[8] Walker, J. C., Monteith, J., Jr., and Wellman, F. L. Op. cit.

RESULTS

CULTIVATED CABBAGE

The results secured with cabbage varieties are divided into two groups, those used commonly in the United States and those used chiefly in Europe. In Table 1 is found, first, the behavior of four varieties of cabbage selected for resistance. It will be seen that little severe injury was noted, although a small percentage of each variety showed some evidence of the disease. Second, the results are given of tests with a number of lots of Volga or Stonehead and Houser, the two varieties earlier noted by Jones and Gilman [9] to possess some natural resistance. With the exception of one lot of Houser, all of these are fairly resistant, although less so than the varieties selected for resistance. Third, the reactions of a number of the varieties more commonly used in America are shown. All those, in contrast with the other two groups, are very susceptible and quite obviously of no value for use upon infested soil.

TABLE 1.—*Comparative Fusarium resistance of a number of cabbage varieties commonly used in the United States, including varieties selected for resistance*

Degree of resistance	Variety	1925 trials: Total number of plants	1925 trials: Plants dead or severely yellowed	1925 trials: Plants slightly yellowed or recovered	1926 trials: Total number of plants	1926 trials: Plants dead or severely yellowed	1926 trials: Plants slightly yellowed or recovered
			Per cent	*Per cent*		*Per cent*	*Per cent*
Resistant (selected)	Wisconsin Hollander	100	1	6	25	0	20
	Wisconsin All Seasons	100	1	7	50	0	6
	Wisconsin Brunswick	50	0	16	25	0	16
	Maryland Flat Dutch	32	0	4	25	0	8
Moderately resistant (natural) [a]	Volga	25	16	36	-----	-----	-----
		-----	-----	-----	24	8	33
		-----	-----	-----	15	0	47
		-----	-----	-----	23	13	13
	Early Stonehead [b]	-----	-----	-----	24	21	29
	Late Stonehead [b]	-----	-----	-----	34	21	41
	Houser	25	28	32	-----	-----	-----
		-----	-----	-----	25	16	24
		-----	-----	-----	24	54	21
		-----	-----	-----	24	17	46
Susceptible	Jersey Wakefield	72	65	17	-----	-----	-----
	Charleston Wakefield	246	64	20	-----	-----	-----
	Copenhagen Market	251	78	9	44	73	0
	All Head Early	112	54	37	51	61	14
	Succession	-----	-----	-----	192	51	27
	All Seasons	-----	-----	-----	47	56	23
	Danish Ballhead	191	73	23	65	65	12
	Haco (red)	256	79	12	246	58	13

[a] Samples of each from several sources.
[b] The Stonehead variety is very similar to if not identical with the Volga.

In Table 2 are given the data from trials of a number of varieties seldom used in America but commonly listed by seedsmen in one or another part of the Old World. Most of these varieties came from England and France but two came from Egypt. Most of them differ from the varieties in general use in America and in the north of Europe in that they produce loose rather than compact heads. The 1925 trials showed many of them to be very susceptible, and only those showing distinct resistance were tested again in 1926. They are

[9] JONES, L. R., and GILMAN, J. C. Op. cit.

grouped roughly into three lots. In the first group are those that are very susceptible and fall into a class with the average commercial variety used in America. In the second group are 12 varieties that are moderately resistant. Many of these compare favorably with the Houser and Volga varieties. In the third group are 7 varieties that are rather highly resistant and could be used with commercial success on infested soil. Though all are not quite in a class with the varieties selected for resistance (Table 1), St. Denis and Vaugirard d'Hiver compare very favorably with them. None of these varieties, however, are of the type suitable for culture in America.

The evidence is sufficient to show that there is a wide range of resistance among cabbage varieties that have been developed, so far as is known, out of contact with the yellows organism. If it is true that cultivated cabbage has evolved from the present known wild form without opportunity for natural selection for resistance to *Fusarium conglutinans*, the conditions found are what might be reasonably expected, since, as is shown later in this paper, the wild form is apparently heterozygous for the resistance character. It is perhaps a mere coincidence that those cultivated varieties of cabbage that became popular in American culture were of the very susceptible sorts, while certain others that might have been introduced except for other undesirable features would not have been jeopardized by yellows.

TABLE 2.—*Comparative Fusarium resistance of a number of cabbage varieties in general use in the Old World but seldom grown in the United States*

Degree of resistance	Variety	Source of seed	1925 trials			1926 trials		
			Total number of plants	Plants dead or severely yellowed	Plants slightly yellowed or recovered	Total number of plants	Plants dead or severely yellowed	Plants slightly yellowed or recovered
				Per cent	*Per cent*		*Per cent*	*Per cent*
Very susceptible	Joanet Hatif	France	24	88	0			
	All Heart	England	24	63	4			
	Offenham	do	25	60	0			
	Express	do	25	52	28			
	Late Drumhead Savoy	do	25	52	20			
	Etamps	France	25	52	12			
	Charentais Tardif	do	24	50	29			
	Blanc d'Hiver	do	24	50	17			
	Milan Cressonier	do	25	48	40			
	Joanet Gros	do	25	48	8			
	Flower of Spring	England	24	42	21			
Moderately resistant	Coeur de Boeuf de Jersey	France	25	36	32			
	Bacalan Hatif	do	26	35	15	28	29	18
	Dax	do	25	32	20			
	Sultani	Egypt	24	29	21			
	Christmas Drumhead	England	21	29	14	24	0	50
	Auvergne	France	24	25	33			
	Earliest of All	England	25	24	16	15	0	0
	Quintal	France	25	24	20			
	Amchiri	Egypt	23	22	0	20	20	10
	Habas	France	25	16	28	21	5	52
	York	do	25	4	12	21	19	14
	Fumel	do	25	12	12	24	21	29
Rather highly resistant	Imperial	England	25	8	28	24	8	42
	Coeur de Boeuf Gros	France	25	8	8	12	8	25
	Pisé	do	25	4	28	14	0	7
	Quintal d'Alsace	do	24	4	21	25	0	12
	Bacalan Gros	do	24	4	17	23	0	9
	St. Denis	do	24	4	0	24	8	8
	Vaugirard d'Hiver	do	25	0	0	22	5	0

WILD CABBAGE

A small quantity of seed of wild cabbage was secured from Sutton & Sons, Reading, England. The first trial was made in 1924 on naturally infested soil. Of the 17 plants grown, 1 showed slight symptoms of yellows. Seed was obtained in the greenhouse the following winter from 3 of the surviving plants, each of which was self-pollinated. The strains thus secured were labeled WC–1s, WC–2s, WC–3s. Results of trials with these lots in 1926 and 1927 are given in Table 3. It is evident that most of these progenies exhibited a fair degree of resistance to yellows and that they were

FIG. 1.—Cabbage and related forms growing on soil thoroughly infested with the yellows organism, Kenosha County, Wis. Photographed September 6, 1927. The bamboo stakes were used to mark each plant as the disease appeared. A, Danish Ballhead cabbage which showed 95 per cent total infection and 68 per cent killed or severely diseased; B, Hartman's Special cauliflower which showed 21 per cent infection but only 6 per cent dead or severely diseased; C, Large White Mammoth broccoli which showed 18 per cent infection, in all cases very slight; D, Improved Danish Brussels sprouts which showed 28 per cent infection, though only 10 per cent were severely affected. See further data in Table 3 and in text

more resistant than the average variety of cultivated cabbage. These data should be compared with those secured from the varieties of cultivated forms.

RELATIVES OF CULTIVATED CABBAGE

Some preliminary tests were made with cabbage relatives in 1924 and 1925. Only a few varieties were used, and the data collected are not included, since they are confirmed by the more extensive trials of

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1926 and 1927. It was noted during the first two seasons that cauliflower, broccoli, and Brussels sprouts were distinctly more resistant than most cabbage varieties, while kohl-rabi, collard, and kale, with the exception of Siberian kale, which was highly resistant, showed varying degrees of susceptibility. The trials of 1926 and 1927 gave opportunity for more extensive analyses. A portion of the 1927 trial plot is shown in Figure 1.

TABLE 3.—*Comparative Fusarium resistance of varieties of the cultivated and the wild forms of Brassica oleracea*

Common name	Subspecies	Variety or strain	1926 trials: Total number of plants	1926 trials: Plants dead or severely yellowed	1926 trials: Plants slightly yellowed or recovered	1927 trials: Total number of plants	1927 trials: Plants dead or severely yellowed	1927 trials: Plants slightly yellowed or recovered
				Per cent	*Per cent*		*Per cent*	*Per cent*
Cabbage	Capitata	Copenhagen Market [a]	44	73	0	149	36	52
		Hollander [a]	65	65	12	104	68	27
		Wisconsin All Seasons [b]	49	0	6	100	0	6
Wild cabbage	Sylvestris	WC–1s	23	0	26	20	25	10
		WC–2s	8	13	0	18	17	17
		WC–3s	25	4	12	60	8	7
Kale	Acephala	Tall Green Curled	21	33	67	73	27	45
		Dwarf Green Curled	25	60	40	46	67	33
		Excelsior Moss Curled	20	25	75	50	36	44
		Thousand-Headed	23	13	26	50	14	2
		Mosbach Winter	17	18	29	50	40	22
		Siberian	24	0	8	75	0	3
Collard	do	North Carolina Short Stem.	21	14	24	50	22	10
		White or Cabbage	24	13	42	49	8	6
		True Georgia	25	24	20	50	14	10
Kohl-rabi	Caulo-rapa	Short Top Early Erfurt	17	65	24	50	36	32
		Early Purple Vienna	20	40	50	50	40	16
		White Vienna	24	38	33	35	20	3
		White Goliath	21	33	52	40	15	25
Brussels sprouts	Gemmifera	Improved Danish	23	0	35	49	10	18
		Paris Market	20	0	50	50	2	18
		Dwarf Improved	18	0	28	49	2	10
		Improved Long Island	26	4	27	50	2	2
		Paris Half Dwarf	25	0	40	-----	-----	-----
		Amager Market	19	0	32	-----	-----	-----
Cauliflower	Botrytis	Hartman's Special	21	0	52	50	6	15
		Vaughan's New Snowball	23	30	30	23	4	30
		Danish Snowball	24	50	38	47	15	36
		Vaughan's Snowball	35	11	63	23	0	39
		Wieboldt's Express	23	13	43	47	6	64
		Extra Early Paris	20	5	85	25	4	76
		Half Early Paris	25	12	52	25	24	48
		Extra Early or Second Erfurt.	24	33	42	50	8	68
		Large Early Erfurt	25	16	56	23	4	61
		Le Normand's Short Stem	25	12	76	25	4	44
		Dry Weather	22	14	55	25	0	36
		Danish Perfection	22	23	45	-----	-----	-----
		Autumn Giant	21	0	0	49	2	10
Broccoli	do	Purple Cape	23	0	13	26	4	4
		Large White Mammoth	25	12	76	55	0	18
		Early Large White French	25	0	0	-----	-----	-----
		White Cape	25	4	68	-----	-----	-----

[a] Susceptible. [b] Resistant.

The results of trials with six varieties of Brussels sprouts are given in Table 3. It should be noted that very few plants were severely affected with yellows, although an appreciable number of each variety showed slight symptoms. The damage was of minor consequence from the commercial standpoint. It is evident that any of these

strains could be grown upon thoroughly infested soil with confidence in their successful evasion of the disease. This subspecies is the most uniformly resistant of any of the groups studied.

Cauliflower varieties were, as a rule, distinctly resistant to yellows, though not generally to such a high degree as Brussels sprouts. It will be seen from the data in Table 3 that in most varieties tested a comparatively large percentage of the plants showed evidence of the disease. The important distinction to be noted is that a large portion of the affected plants were only slightly attacked, and in many cases the actual damage to a variety as a whole was relatively small. (Fig. 1.) But here again, as in cabbage, varieties differ widely in their reactions. It is of interest to note particularly the wide variation among the different strains of Snowball. Undue reliance should not be placed upon a single test for a given variety, for there might exist numerous strains of that form which vary considerably in resistance. As the practical need for resistant strains of cauliflower arises, advantage should be taken of the natural yellows resistance both for its value in meeting needs temporarily and as a basis for further improvement.

Broccoli, which is very closely related to the cauliflower, probably less often encounters yellows, as it is most commonly grown as a winter crop. Curiously, however, all four varieties tested are highly resistant to *Fusarium conglutinans*, and there will probably be little need for their further improvement in this respect.

Many varieties of kale have come into use and are widely grown. Only a few of those most common in America were tested. It was found (Table 3) that the curly-leaved varieties were decidedly susceptible. The smoother leaf types such as Thousand-Headed and Mosbach Winter were on the whole more resistant, and the Siberian was very resistant. The collards are closely related to the smooth-leaved kales and are especially popular in the southern part of the United States. They were susceptible to about the same degree as Thousand-Headed and Mosbach Winter kales. Thus, as in the forms previously considered, there is considerable variation in resistance within this subspecies. The smooth-leaved forms were only moderately damaged on infested soil under conditions favorable for the disease, while curly-leaved varieties were so affected that their commercial value was seriously impaired. In any of the smooth-leaved varieties considered except Siberian there is ample opportunity to develop more highly resistant strains through selection. In the curly-leaved forms the possibility of finding resistant individuals for selection is not so certain, but in spite of the fact that all three of these varieties showed 100 per cent yellows in the 1926 trials, a few plants were resistant in two of the varieties in 1927.

Kohl-rabi appears to be the most generally susceptible subspecies with the exception of cabbage. (Table 3.) All four varieties tested were severely attacked. From their behavior it is evident that they can not be safely used on thoroughly infested soil during the season favorable for the development of yellows. Further search might possibly reveal more naturally resistant strains. On the other hand, it is evident that the way is open for improvement in resistance through selection from any of these commercial varieties by methods already shown to be successful with cabbage.

DISCUSSION

Brassica oleracea is interesting for study of the variation in resistance to *Fusarium conglutinans* among its cultivated subspecies and varieties. Whether or not the present wild form of Europe is the progenitor of our cultivated forms or whether it also has evolved from some common ancestor is problematical. The wild form of cabbage as we now know it, when tested on soil infested with *F. conglutinans*, shows a high degree of resistance, but a study of the progeny of individual plants shows them to be segregating in this character. In fact, of 154 plants tested from three selfed progenies, 33 became diseased, showing a segregation reasonably close to 3 resistant to 1 susceptible. This is in accordance with previous findings in the case of cultivated cabbage, where resistance behaves as a single dominant Mendelian character.[10]

The apparent absence of the Fusarium disease in Europe, even in regions climatically favorable for its development, seems to substantiate the belief that the parasite is of American origin. The development of the subspecies of *Brassica oleracea* now known and their further segregation into numerous varieties under cultivation have thus gone on out of contact with this disease. If the yellows organism had been present and active it is not unlikely that natural elimination of susceptible individuals of this species, which normally is cross-pollinated, would by this time have resulted in quite general resistance. On the contrary, the state of affairs that did actually exist would naturally lead to no uniformity in resistance. Chance might result in certain varieties becoming highly resistant and in others becoming very susceptible.

The data that have been presented show this to be the case. The cabbage varieties range from the highly resistant types such as St. Denis and Vaugirard d'Hiver to the very susceptible forms such as Danish Ballhead. In kale the very resistant Siberian stands at one extreme and the susceptible moss-curled types at the other. All Brussels sprouts varieties tested are highly resistant, while all kohl-rabi forms examined are very susceptible. Out of this whole series, however, the important fact is revealed that no variety tested, with the possible exception of one of the moss-curled kales, appeared to be homozygous in the susceptible character. Thus since resistant individuals occur the opportunity exists to improve by selection any given variety in this respect, as has been already amply demonstrated with cabbage.

The observations here reported are not intended as a final statement of the comparative behavior of the subspecies and varieties mentioned. Since the tests were made in a single locality, the results must be taken only as an indication of the relative resistance of the varieties used. However, experience with resistant strains of cabbage has led to the conclusion that the tests thus employed give a fair index to behavior to be expected in other localities, granted the general assumption that the rise in the average temperature may be expected to increase the incidence of yellows. Variation with locality in the pathogene leading to selective pathogenicity has not yet come to the attention of the writers, but should be constantly watched for as a possible complicating factor.

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[10] WALKER, J. C. STUDIES UPON THE INHERITANCE OF FUSARIUM-RESISTANCE IN CABBAGE. (Abstract) Phytopathology 16: 87. 1926.

SUMMARY

A survey was made of the behavior of wild cabbage and a number of varieties of the cultivated subspecies of *Brassica oleracea* when grown upon soil infested with *Fusarium conglutinans*.

Although most cabbage varieties commonly used in America are very susceptible to yellows, various degrees of resistance were found when a number of European varieties not ordinarily used in America were tested.

The wild cabbage of Europe was highly resistant, but selfed progenies from individual plants showed about one-fourth of the plants diseased.

Brussels sprouts and broccoli varieties, though showing a considerable number of plants slightly affected, were not seriously damaged by yellows.

Cauliflower varieties varied somewhat in reaction, but in general they were damaged to a greater degree than broccoli or Brussels sprouts.

The kale varieties differed widely in susceptibility. The Siberian kale was very resistant, while the curled-leaf types were very susceptible. The smooth-leaf varieties and the collards occupied an intermediate position.

The kohl-rabi varieties were all very susceptible.

Within all of the forms tested a sufficient number of individuals survived to make it possible to improve their resistance through selection.

THE ISOLATION OF THE FUNGUS THAT CAUSES CITRUS MELANOSE AND THE PATHOLOGICAL ANATOMY OF THE HOST [1]

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INTRODUCTION

Citrus melanose[4] is characterized by the presence of small, pustular lesions that look like drops of caramelized sugar on leaves, twigs, and fruits. These lesions are initiated while the parts are still young, and at maturity they may occur as isolated dots or may be arranged in streaks, rings, or extensive irregular patches. The fungus that causes melanose possesses two stages, a pycnidial one, *Phomopsis citri* (*2*),[5] described in 1912, and an ascigerous one, *Diaporthe citri* (*10*), described in 1926.

Melanose was first described in 1896 by Swingle and Webber (*8*), who first observed it in November, 1892, at Citra, Fla. They were unable to establish the cause definitely, but assumed it to be a fungus. Furthermore, until now, no one has succeeded, even after repeated attempts, in isolating the causal organism from melanose lesions. The pathogene has been isolated repeatedly, however, from dead twigs and from fruits affected with stem-end rot, two manifestations of the disease that can not properly be designated melanose, but that result from infection by the same organism. The relationship between stem-end rot and dying back of twigs has been determined by the investigations of Fawcett (*2*) and the relationship of melanose to the other two manifestations by Stevens (*7*) and by Floyd and Stevens (*4*).

Fawcett (*2*) isolated *Phomopsis citri* from the interior of fruits affected with stem-end decay and from the interior of dead twigs. He showed by the cultural similarity of isolations from these two sources and by inoculations that these two forms of the disease are caused by the same fungus.

Floyd and Stevens (*4*), who did not suspect at first that melanose and stem-end rot were related, later found that the two forms were undoubtedly caused by the same fungus. They occasionally found particles of fungous hyphae in melanose lesions, but were not able to demonstrate whether these were parts of the causal organism or of some secondary invader. They concluded from their microscopic examination of lesions of different ages that no bacterial or fungous organism that could be considered a cause of the disease could be found connected with the affected tissues or the adjoining cells. They tried to isolate the causal organism by planting on various media bits of

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[4] This usage of the term "melanose" is in accord with that first employed by Swingle and Webber (*8*) and is employed in the same restricted sense throughout this paper.
[5] Reference is made by number (italic) to "Literature cited," p. 252.

diseased leaves that had been soaked in 1:1,000 mercuric chloride for three to five minutes and washed in sterile water. In nearly every case Colletotrichum overran the cultures and made it impossible to isolate any other fungus that might have been present.

Stevens (7) in 1918 concluded that there was no growth of the fungus within the affected tissues. All attempts to isolate the fungus from artificial inoculations gave negative results, and the organism was never obtained from spots or markings formed naturally. Melanose infections were secured, however, from inoculations with pure cultures of *Diaporthe citri*.

Winston, Bowman, and Bach (9) made fully 1,000 systematic attempts to recover the causal organism from melanose blemishes, but without success in a single instance. Leaf and fruit tissues from both old rough lesions and young, almost invisible spots were cultured. Such surface disinfecting agents as ethyl alcohol, ether, acetic acid, mercuric chloride, and hydrogen peroxide were used, after which the material was rinsed in sterile tap water before being planted in cultures. Repeated attempts were also made to isolate the organism without first subjecting the lesions to surface disinfection.

While the results of previous investigations of melanose, stem-end decay, and the dying back of twigs and branches have left no reasonable doubt as to the identity of the fungus that causes them, the failure to isolate the pathogene from melanose lesions has made it impossible to fulfill completely Koch's postulates. The present study is, therefore, concerned both with the isolation of the pathogene from melanose lesions and with the pathological anatomy of the host, to which no special attention has been given except by Floyd and Stevens (4). The results show that it is possible to complete the rules of proof of pathogenicity of the fungus that causes melanose, and the findings and interpretations relative to anatomical changes that are herein recorded are believed to contribute further to an adequate understanding of the disease.

ISOLATION

Young artificially inoculated orange leaves were used in the preliminary trials. These leaves had been inoculated eight days previously with suspensions of conidia from pure cultures. The young lesions, which were plainly visible at this time, were excised, dipped in 95 per cent alcohol, flamed, and placed on slants of 2 per cent potato-dextrose agar. The resultant mycelial growth in 7 of the 10 slants presented the characteristic appearance of *Diaporthe citri*, while no growth occurred in the other three tubes. This was so unusual in the light of previous experience that subcultures were made on sterilized stems of pigeon pea (*Cajanus indicus*), a substratum favorable for pycnidial formation, in order to induce the development of fruiting bodies. Conidia that were typical of the pycnidial stage, *Phomopsis citri*, were produced in due time in these subcultures. Additional proof of this identity was secured by the inoculation of young grapefruit leaves with these cultures. Infection resulted and typical melanose markings developed.

As a consequence of the successful isolation of the pathogene in this preliminary trial, further attempts were made to obtain the melanose fungus in culture from lesions of varying ages on leaves,

twigs, and fruits. In all cases the surfaces of the affected parts were disinfected by dipping them in 95 per cent alcohol and removing the alcohol by flaming. The lesions were then excised and placed on agar plates or slants. As soon as growth had proceeded sufficiently, which usually required four or five days, subcultures were made from portions of the colonies that looked like the melanose fungus. In a number of cases, especially when young lesions were used, the fungus appeared in pure culture in the planted plates or tubes and it was not necessary to make subcultures. As a routine practice, however, the fungus can not be isolated without making subcultures so as to separate the pathogene from the various secondary invaders that early overgrow the cultures. It is possible that the failure of other investigators to isolate the melanose organism is due in part at least to their failure to use subcultures, and in consequence the causal fungus was crowded out or intermingled with those fungi that grew more rapidly.

In order to test the efficacy of this method of surface disinfection, two normal grapefruit leaves were immersed on June 29, 1926, in a suspension of conidia of the Phomopsis stage. After they had dried one was dipped in alcohol, flamed, and bits of the leaf tissue were planted on agar. Fragments of tissue from the other were planted without disinfection. Of six plantings made from each leaf no growth appeared in any from the first leaf, while three cultures of the melanose organism and three of other species of fungi were obtained from the second. This test was repeated on July 12, 1926, when six plantings were again made from a disinfected leaf and six from one that was not given surface disinfection. Again no growth appeared in the first case, and the second yielded two cultures of *Diaporthe citri* and four of other organisms. A third trial made in the same manner on March 18 employed 20 plantings of leaves that were given surface disinfection and an equal number that were not disinfected. No fungous growth appeared around any of the plantings from disinfected leaves, whereas 14 cultures of the melanose fungus were recovered from those that were not disinfected, 5 yielded other fungi, and the other remained sterile. This method of surface disinfection is therefore regarded as effective, at least for comparatively normal leaf tissue. That it is also highly effective for tissues with minute fissures is indicated by the results given later. (Table 3.)

During the course of this investigation several additional tests of the effectiveness of this method of surface disinfection were made, employing leaves and twigs that were free from melanose lesions and apparently normal but on the surface of which the conidia may reasonably be presumed to have been present. The results are summarized in Table 1.

No cultures of the melanose organism were secured in this series of 108 trials, although 4 yielded *Colletotrichum gloeosporioides*, a fungus that appears always to be present on citrus throughout Florida. It seems improbable that the conidia of this Colletotrichum would survive disinfection and at the same time those of the melanose fungus be destroyed. It may be that the tissues were only apparently normal and that the fungus had established itself in minute fissures.

TABLE 1.—*Results of experiments to isolate Diaporthe citri from apparently normal tissues*

Source of material	Date of experiment	Number of plantings made	Number of plantings yielding— Diaporthe citri	Number of plantings yielding— Miscellaneous organisms only	Number of plantings remaining sterile
Old orange leaves	Oct. 31, 1925	9	0	0	9
Mature grapefruit leaves	do	6	0	0	6
Leaves of Chaetospermum glutinosum	Nov. 7, 1925	6	0	0	6
Mature orange leaves	do	14	0	1	13
Leaf scars on orange twigs	Nov. 15, 1925	15	0	0	15
Leaves of oranges from June flush	June 15, 1926	16	0	1	15
Orange twigs of spring growth	June 19, 1926	12	0	0	12
Old grapefruit leaves	July 12, 1926	11	0	0	11
Mature grapefruit leaves	June 15, 1927	19	0	2	17
Total		108	0	[a] 4	104

[a] Colletotrichum gloeosporioides.

The attempts to isolate *Diaporthe citri* from melanose lesions have extended over a period of three seasons. Use has been made of lesions on leaves, twigs, and fruits of orange and grapefruit, on leaves of tabog (*Chaetospermum glutinosum*), on twigs of Mexican lime, and on fruits of faustrime (Mexican lime × Australian finger lime). Table 2 contains a summary of the essential features and results of these isolation experiments.

A total of 115 cultures of the melanose fungus have been obtained from 506 plantings of melanose lesions. These lesions varied in age from 6 days to approximately 9 months. Those of definitely known age were obtained by artificial inoculation or occurred following rains on April 8, 1926, and February 14, 1927. The age of the other natural infections was estimated from the age of the flush of growth on which they occurred.

It is of special interest to note, too, that cultures from lesions that had not yet advanced to the stage in which the cuticle had become fissured yielded the pathogene in pure culture or else remained sterile. In general, a larger proportion of successful attempts resulted from isolations from young lesions than from old ones, since various other fungi, primarily *Colletotrichum gloeosporioides* and secondarily *Diplodia natalensis*, were always present in mature lesions. In no case was the percentage of successful attempts to isolate *D. citri* from melanose lesions as large as usually results from attempts to isolate other plant pathogenes from other host tissues. One probable reason for the relatively small number of successful attempts to isolate the melanose fungus is, as will be shown subsequently, that the infected tissues are flooded with gum, which may envelop the mycelium and prevent it from growing out of the tissues in culture. Furthermore, as will be shown in this paper, anatomical studies reveal the fact that the host cells are disintegrated by enzymes. A concomitant digestion of the hyphae of the pathogene may therefore be expected.

TABLE 2.—*Results of experiments to isolate Diaporthe citri from melanose lesions*

Approximate age of lesions	Source of material	Type of infection	Date of experiment	Number of plantings made	Number of plantings yielding— Diaporthe citri	Number of plantings yielding— Miscellaneous organisms *only*	Number of plantings remaining sterile
6 days	Young grapefruit leaves	Artificial	May 23, 1926	6	2	0	4
Do	do	do	June 15, 1926	15	4	2	9
8 days	do	do	Oct. 17, 1925	10	7	0	3
10 days	Grapefruit leaves	Natural	Feb. 24, 1927	24	5	7	12
15 days	Orange twigs	Artificial	July 12, 1926	8	1	0	7
Do	Grapefruit leaves	do	July 27, 1926	11	4	3	4
21 days	Lime twigs	Natural	June 30, 1927	19	4	0	15
27 days	Young orange fruit	do	May 5, 1926	6	2	1	3
34 days	Lime twigs	do	July 11, 1927	16	8	8	0
Do	Young grapefruit fruit	do	May 12, 1926	15	6	5	4
42 days	*Grapefruit leaves*	*do*	*July 12, 1926*	*15*	*2*	*1*	*12*
Do	Orange leaves	do	July 16, 1926	12	1	11	0
Do	Leaves of Chaetospermum glutinosum.	do	Oct. 20, 1925	10	2	6	2
44 days	Grapefruit leaves	do	July 22, 1927	23	4	4	15
45 days	Orange fruit	do	May 12, 1926	20	5	11	4
49 days	Grapefruit leaves	do	July 27, 1926	7	1	0	6
63 days	do	do	June 11, 1926	8	2	3	3
Do	Orange fruit	do	do	3	2	0	1
Do	Grapefruit twigs	do	do	6	1	0	5
70 days	Grapefruit fruit	do	Aug. 12, 1926	11	1	10	0
71 days	Orange leaves	do	June 19, 1926	12	3	6	3
80 days	Orange fruit	do	June 28, 1926	6	1	0	5
Do	*Grapefruit leaves*	*do*	*do*	*10*	*4*	*2*	*4*
84 days	Orange leaves	do	Dec. 17, 1925	6	2	1	3
87 days	Grapefruit leaves	do	June 15, 1927	23	4	1	18
91 days	Orange leaves	do	Sept. 11, 1926	20	7	2	11
94 days	Grapefruit leaves	do	June 22, 1927	22	4	5	13
Do	do	do	July 12, 1926	24	5	7	12
Do	Grapefruit twigs	do	do	9	1	8	0
Do	Faustrime fruit	do	July 16, 1926	8	1	4	3
103 days	Orange leaves	do	Dec. 11, 1925	6	2	1	3
130 days	Grapefruit fruit	do	Aug. 16, 1926	12	1	11	0
131 days	Orange fruit	do	Aug. 17, 1926	27	3	16	8
149 days	Faustrime fruit	do	Sept. 4, 1926	11	1	5	5
152 days	Orange fruit	do	Sept. 7, 1926	6	0	4	2
167 days	*Grapefruit leaves*	*do*	*Sept. 22, 1926*	*8*	*1*	*7*	*0*
179 days	do	do	Oct. 4, 1926	18	2	14	2
183 days	do	do	Oct. 8, 1926	10	1	1	8
270 days	do	do	June 11, 1926	13	7	1	5
278 days	do	do	June 19, 1926	10	1	0	9
Total				506	115	168	223

The reisolation of the melanose fungus from infections resulting from artificial inoculation makes it possible to complete, for the first time, Koch's rules of proof of pathogenicity.

Although young lesions yielded the melanose pathogene in pure culture, and its presence in mature lesions is presumably due to the fact that the mycelium remains alive from the time of primary infection, this may not necessarily be the case. Additional evidence on this point comes from the planting of lesions resulting from mechanical injuries and from attacks of the rust mite (*Phyllocoptes oleivorus* Ashm.). The data on these tissue plantings are assembled in Table 3.

TABLE 3.—*Results of experiments to isolate Diaporthe citri from various lesions on grapefruit other than melanose*

Source of material	Date of experiment	Number of plantings made	Number of plantings yielding		Number of plantings remaining sterile
			Diaporthe citri	Miscellaneous organisms only	
Abrasions from rubbing on fruit	May 12, 1926	10	0	0	10
Greasy melanose on leaves of September, 1925	June 11, 1926	10	1	4	5
Abrasions on fruit from contact with limbs	June 15, 1927	25	0	19	6
Russeted fruit from rustmite injury	do	20	1	16	3
Russeted fruit	June 22, 1927	21	0	2	19
Abrasions on fruit from rubbing	do	22	0	2	20
Abrasions on fruit	June 30, 1927	24	1	9	14
Abrasions on June bloom fruit	July 11, 1927	12	0	12	0
Rust-mite russeted fruit	do	12	0	10	2
Russeted fruit	July 22, 1927	21	1	12	8
Total		177	4	86	57

Four of the 177 trials in this series yielded cultures of the melanose fungus, and *Colletotrichum gloeosporioides* again predominated among the miscellaneous organisms present. In the light of the previously mentioned results on the effectiveness of the method of surface disinfection, it seems improbable that these cultures originated from conidia that were present on the surface or were lodged within crevices of the lesions. As is well known, not only Colletotrichum but also the melanose fungus can occupy tissues saprophytically. The dead-twig manifestation of the disease, in the case of the latter organism, is evidence of this condition. It seems more reasonable, therefore, to believe that the colonies resulted from mycelia within the tissues. This is substantiated by microscopic examination of russeted and abraded citrus tissues, which reveal the universal presence of hyphae within them.

PATHOLOGICAL ANATOMY

Collections of material from natural infections that occurred during April and June were used in studying the anatomy of lesions in advanced stages of development. Infections resulting from artificial inoculations with pure cultures were employed in studying the early stages of the disease. The inoculations were made by wetting bits of absorbent cotton in suspensions of conidia and placing them upon tender leaves and fruits of grapefruit. These were then protected against desiccation by being wrapped in waxed paper. Both free-hand and paraffin sections stained in Haidenhain's iron-alum haematoxylin were used. Infection is effected 36 to 48 hours after inoculation by direct penetration of the upper epidermis. This phenomenon can best be observed in free-hand sections cut parallel to the leaf surface. The germ tube penetrates the cuticle and passes downward between the lateral walls of adjacent epidermal cells. (Fig. 1, A.) Thence it branches and extends intercellularly between the palisade parenchyma.

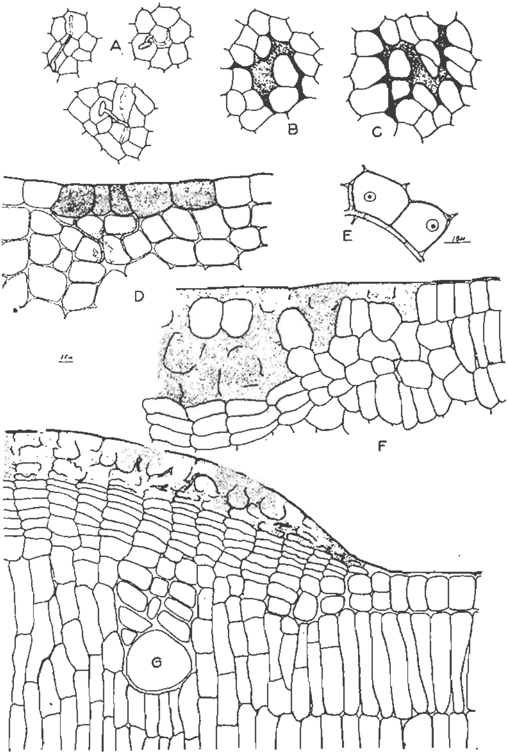

FIG. 1.—A, surface views of grapefruit leaves showing penetration of conidial germ tubes into vertical walls of epidermal cells, 36 hours after inoculation. B, surface view of a grapefruit leaf bearing a melanose lesion 80 hours old, showing gelatinization of cell walls. C, lesions on grapefruit leaves 110 hours old, with the dissolution of primary cell membranes, and the resultant formation of free-floating cells. D, margin of a melanose lesion 80 hours old on young fruit in vertical section. The center of the lesion is sunken, the cells are collapsed or filled with gum, and the hyphae are intercellular. E, intercellular hypha of *Diaporthe citri*. F, vertical section of a lesion 7 days old showing dissolution of cell walls with resultant gummosis and the beginning of the formation of the suberized layer. G, mature melanose lesion in which the corky layer has completely separated the affected tissues from the subjacent normal tissues. The cuticle has been ruptured by tensions and the gum mass has become brown. (A, B, C, D, F, and G were drawn to the scale shown below D, and E to the scale at its right)

There is no evidence of infection visible to the unaided eye until the fourth day after inoculation. At this time the epidermal cells and intercellular spaces are filled with a gummous substance that gives a bright-red precipitate when treated with hydrochloric acid and phloroglucin. This gummous substance is manifestly a hemicellulose derivative resulting from the digestion of the cell wall by pectic enzymes. That such enzymes are present is shown by cultures of the melanose fungus on pectin agar, which was made by the addition of pectin to plain agar. Pectin from two sources was used, a commercial lemon pectin in powdered form and a commercial apple pectin purified by repeated precipitation with alcohol. The initial reaction of the media was adjusted to approximately pH 5, and methyl red was added as an indicator. The color disappeared in a broad area surrounding the colonies, which indicated an increase in alkalinity as a result of the growth of the fungus. A narrow clear zone formed at the borders of the colonies resulted from the digestion of the pectin by enzymes.

It is apparent from examination of lesions four to five days after inoculation that the primary cell membranes are involved in gummous degeneration and that the accumulation of gum between cells forces them apart. Thin-walled cells floating free in the gum mass can be seen at this stage. (Fig. 1, B and C.) The degeneration of the inner lamellae of the walls of these free-floating cells proceeds centripetally until the cell contents are freed and become mixed with the gum matrix. The dissolution and collapse of cells results in the formation of a depression, which marks the site of the lesion. The cuticle, however, remains intact. This is shown by the microscopic appearance of both free-hand and paraffin sections. (Fig. 1, D.) It is shown indirectly by the absence of secondary invaders from isolations from lesions 6 to 7 days old, whose surfaces have been disinfected, since such isolations either have yielded the melanose fungus alone or have remained sterile.

By the time the lesions are 7 days old the differentiation of a phellogen layer has begun in an area several cell layers in advance of gummosis. This is manifest by the formation of cell walls in a plane parallel to the leaf surface. (Fig. 1, F.) The epidermal cells and any of the subepidermal tissues may be involved in the formation of the suberized layer. As a result, a saucer-shaped suberized tissue which completely separates the invaded normal tissues, is formed between them. The growth of this corky tissue proceeds until the tiers of cork are 7 to 12 cell layers in thickness. Meanwhile the normal growth of the healthy tissues beneath the lesions results in everting the corky layer and thus in raising the lesion so that it protrudes about the surface. (Fig. 1, G.) At this stage when the lesions are abundant the affected parts are rough to the touch like sandpaper.

Coincident with the development of the corky layer the tensions on the cuticle result in its rupture and the gum mass on exposure to the air becomes brown and dry. This permits various fungi to penetrate through the fissures. The necrotic tissues of old lesions yield in culture not only the melanose fungus but also species of Colletotrichum, Gloeosporium, Fusarium, Pestalozzia, Cladosporium, and Alternaria.

DISCUSSION

The foregoing observations on penetration and on the presence of the melanose pathogene within the tissues are at variance with previously published accounts. Floyd and Stevens (*4*) stated that there was apparently no vegetative growth of the fungus within infected tissue in causing the formation of melanose lesions, as evidenced by the fact that stained sections failed to reveal mycelium either within diseased tissues or within adjoining cells. They suggested that the lesions may possibly be caused by some chemical substance or toxic principle that is eliminated by the germination or death of the conidia. In support of this view Stevens (*7*) reported that dilute lemon juice, when sprayed on young foliage, caused the formation of markings quite typical of melanose. Whatever may be the action of chemicals, the present observations show that the lesions are initiated by the direct penetration of the tissues by the melanose fungus, which accords with infection phenomena in general. However, the observations on the relative abundance of mycelia within melanose lesions, when comparison is made with lesions produced by other pathogenes on other hosts, lead to the conclusion that the hyphae of *Diaporthe citri* are very scarce even in young lesions. The occasional isolation of this fungus from lesions other than melanose markings can most reasonably be interpreted as showing that it may occupy such tissues as a secondary invader.

Microscopic examination discloses the fact that in the formation of melanose lesions the zone of gummous degeneration extends in advance of the mycelium, which shows that this gum is undoubtedly the result of enzymotic action. This observation accords with the well-established fact that the freshly exuded gum in woody plants contains a pectin-dissolving enzyme (*5*) and that the production of gum is due to enzymotic action. While gum formation in citrus [6] may occur as a response to injury from any cause, as evidenced by its occurrence in connection with such diseases as exanthema, psorosis, and foot rot, the proximate cause in the case of melanose is the pathogene itself through its ability to secrete pectic enzymes.

It is not necessary to assume that the dissolution of the cell walls is due entirely to enzymes secreted by the fungus, since Hodgson (*6*) has shown in his studies on abscission of leaves and fruits that the pectic enzymes or their appropriate zymogens exist normally within the tissues of citrus.

The manner of the formation of the corky layer presents no novel features, but appears to correspond in all essentials with cork formation of other plants. This layer is therefore to be regarded as wound cork, which is well known to arise as the normal response to traumata.

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[6] The monograph by Butler (*1*), to which the reader is referred for a comprehensive account of gummosis, contains a review of the numerous investigations on this problem. The more recent studies by Fawcett (*3*) further contribute to an understanding of this phenomenon.

SUMMARY

Investigators have hitherto been unsuccessful in isolating *Diaporthe citri* from citrus melanose. During the present study 115 isolations have been secured from a total of 506 trials. Isolations have been made from leaves, twigs, and fruits from lesions that varied in age from 6 days to approximately 9 months. The isolation of *D. citri* from melanose lesions has made it possible to complete Koch's rules of proof of pathogenicity.

Surface disinfection was accomplished by immersion in alcohol and removal of the alcohol by flaming. The lesions were then planted, and as soon as the pathogene had grown from them it was separated from the secondary invaders by means of subcultures.

Direct penetration of conidial germ tubes has been observed. The mycelium is intercellular, and the tissues are disintegrated in advance of the hyphae.

Two phenomena occur in the formation of melanose lesions—gummosis and suberization. Gummosis is the result of enzymotic action, primarily of pectic enzymes, and the melanose fungus itself is able to secrete these enzymes. Suberization is a wound response of common occurrence in citrus and in many other plants.

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